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GROWTH SUBSTANCE AND ION ABSORPTION

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Introduction. The intake of ions by the plant is a consequence of its reaction to the composition of the medium. As a base from which to start further research into this phenomenon, we suggested a working hypothesis founded upon the following concepts: — The ion absorption is a result of the differences in the physico-chemical activity of the ions of the periphery of the root and the ions of the medium. This difference in ion activity causes a flow of ions to move in the direction of the plant, which flow is kept going by transportation and inactivation of the ions taken up by the plant (pump-function). A complication of the process in question arises from the adsorption of ions from the medium to the surface of the root (surface-function) (Schuffelen and Loosjes[8]), Schuffelen[9]).

Energy is needed for the continuation of the pump-function and this is provided by processes taking place in respiration and in accumulation of organic material. It is consequently a function controlled by the living processes (Hoagland and Steward[3]), Lundegårdh[5])). The surface-function is of a more non-living nature and is regulated by the composition of the plasma of the root wall. This plasma is highly important in connection with the study of the significance of the ion ratio of the medium in relation to plant growth, while the pump-function is related to the ion intake process as a whole.

As plant growth substances play an important rôle in living processes it may be expected that the ion intake will also be influenced by auxin concentrations as suggested by Lundegårdh[6]) and others. Moreover, the fact that Veldstra[10]) considers the growth substances to act as transport (permeability) regulators

encouraged us to examine the influence of some growth substances on ion absorption.

Experimental methods and results. Following Hoagland and Broyer's method [2]) oat plants were grown and, when three weeks old, their roots were cut off and used as test objects. The excised roots were put in glass jars, containing solutions of a constant ion amount and different concentrations of growth substances, and kept there for 8 or 10 hours. The solutions were constantly stirred and aerated by an air flow. After the experiment the root substance was washed with distilled water, dried and weighed for each subject separately and analysed by the flame spectrographic method (Lundegårdh [4]), Schuffelen [7])). The relating experimental data are combined in Tables I and II, and the results are represented graphically in Figures 1 and 2.

TABLE I

Data of the experiments						
Series No	Date of sowing	Date of experiment	Period of absorption hr	Temp. during absorption °C	Average root weight (mg dry matter)	Composition of roots at the beginning (ME p. 100 g dry matter)
1	13 June 1944	7 July 1944	10	21—23	181	17,2 — K 3,8 — Ca
2	4 July 1944	26 July 1944	8	19—21	216	22,9 — K
3	4 July 1944	27 July 1944	8	21—22	222	21,8 — K
4	4 July 1944	28 July 1944	8	20—21	218	19,3 — K

TABLE II

Data of the solutions	
Series No	Composition of the solution (mol per liter)
1	2.10^{-3} KCl + 2.10^{-3} $CaCl_2$ + (0 — 2.10^{-6}) naphtalene (1) acetic acid
2	3.10^{-3} KCl + 1.10^{-4} $CaCl_2$ + (0 — 1.10^{-4}) napthalene (1) acetic acid
3	3.10^{-3} KCl + 1.10^{-4} $CaCl_2$ + (0 — 1.10^{-4}) indol (3) acetic acid
4	2.10^{-3} KCl + 2.10^{-3} NaCl + 2.10^{-3} $MgCl_2$ + (0 — 1.10^{-4}) indol (3) acetic acid

It is evident that the amount of potassium taken in by the roots is independent of the concentration of the growth substance present in the medium so long as this does not exceed 10^{-6} mol per liter. The same holds true for the intake of sodium although here the range of

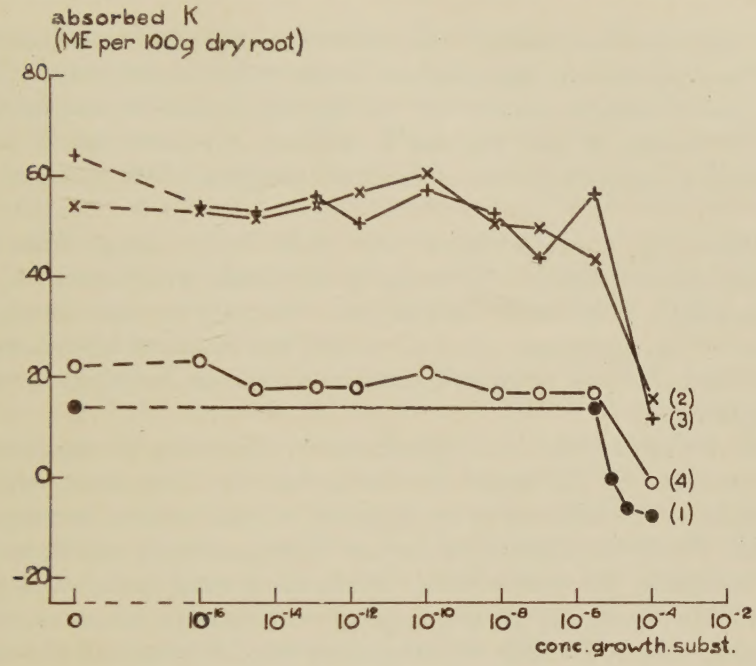

Fig. 1. The influence of the concentration of the growth substance on the potassium intake by excised roots.

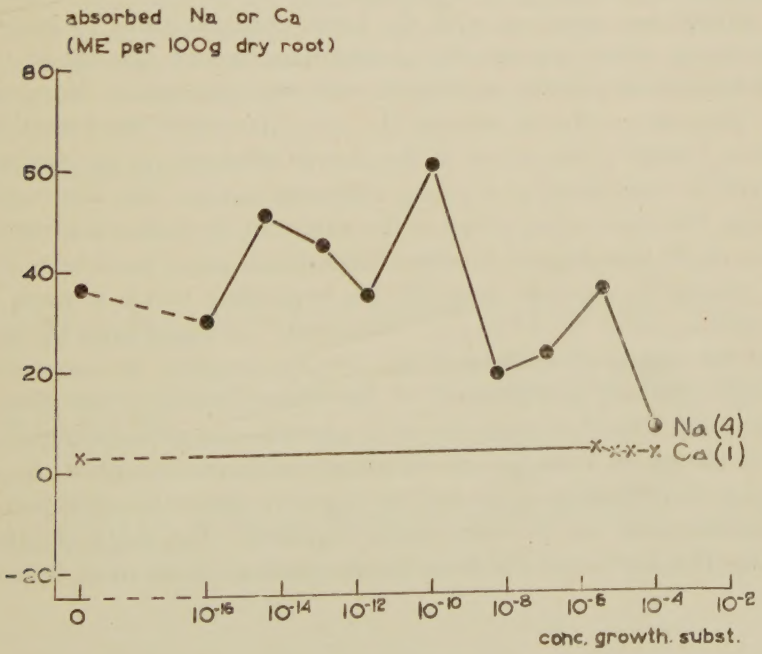

Fig. 2. The influence of the concentration of the growth substance on the sodium and calcium intake by excised roots.

the figures is much greater. The growth substance had no influence, in these experiments, upon calcium intake. A rise in concentration of the growth substance above 10^{-6} mol per liter causes the ion intake to fall rapidly (K and Na) and in one case a negative intake was measured, suggesting a loss of ions from the plant to the medium.

Discussion. Commoner and Mazia [1]), using slices of potato tubers, showed a reduction in ion intake with a solution of hetero-auxin of about 10^{-4} mol per liter. Contrary to their findings, however, no significant rise of ion intake was measured for either of the concentrations of growth substances used on oat roots in our experiments.

In view of Veldstra's hypothesis [1c]) concerning the regulation of transport by the auxins as well as our own hypothesis which emphasises the influence of ion diffusion, we may suggest two hypothesis. Firstly the diffusion of the ions could possibly be accellerated by increasing the permeability (auxin as opening factor) and inhibited by decreasing the permeability of the plasma (auxin as condensing factor). The ions would consequently be influenced directly in their transport by the action of the growth substance. In our opinion, however, it seems unlikely that ion transport should be regulated by compactness of protoplasma. The fact that no increase in uptake was observed with the lower concentrations of growth substances which, presumably should have caused opening of the plasma colloid is not in accordance with this assumption. Moreover the observation that in one case the concentration of the potassium fell as a result of the action of the growth substance in the medium should be considered as a strong argument against this first hypothesis. The condensing action of the auxins in the higher concentrations might have impeded transport but should never have been able to reverse the process. Secondly an hypothesis which is more in accordance with Veldstra's viewpoint [1c]) is based upon the fact that the supply of material to be used by the plant as a source of energy (glucose) is dependent on the concentration of the growth substance. This view seems the more plausible since the independance of ion intake from growth substance in lower concentrations, as well as its inhibiting action and the negative figures found in higher concentrations can be more easily explained. The roots obtained using Hoagland en Broyer's method [2]), are most favour-

able conditioned regarding ion intake which suggests, that glucose is easily transported. Low concentrations of growth substances opening the plasma are obviously not able to improve the transport of glucose and therefore have no effect. High concentrations of growth substances, which condense the plasma impede sugar transport, hampering thereby the pump-function and decreasing ion absorption. Most striking is the effect where the sugar transport is so inhibited that the periphery of the root is not even able to maintain its condition (a low ion activity) and as a result of the smaller supply of energy, loses ions to the medium.

The fact that Commoner and Mazia[1]) in their experiments with slices of potato tubers traced a benificial influence of hetero-auxin in a concentration of about 10^{-6} mol per liter fits exactly this second hypothesis. It should be remembered, however, that slices of potato tubers are not so favourably conditioned regarding ion intake as roots. Therefore, it is justifiable to draw the conclusion that the action of the growth substance on ion intake is no a direct one but takes place via the living process. In this respect sugar transport probably plays an important rôle.

Summary

The results of some experiments concerning the influence of growth substance on ion intake by plants are presented. Is has been found that the rate of ion intake is independent of low concentrations of growth substance. It is suggested that the intake takes place via the living processes. The regulation of the transport of glucose is mentioned as a possible direct function of the growth substance.

Received December 13, 1947

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ANALYSIS OF THE ROOT SAP OF THE BIRCH

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Introduction. In order to find out whether tree roots absorb nutritive substances in the same proportions from the different soil horizons, chemical analyses were carried out on the exuded sap tapped from birch roots at various depths in the ground. The following represents an account of the results obtained. In advance I wish to stress strongly that it is a question only of orientating investigations.

Material and Soil. Most of the roots that were tapped belonged to fourteen young birch trees the heights of which varied between 5.1 and 10.1 m and their diameter between 8.3 and 12.5 cm. The trees were situated near each other overgrowing a young oak plantation in the enclosure of "Fortunen" that lies c. 1 km from the State Afforestation Research District at Springforbi, north of Copenhagen. The trees were sound and of good growth.

The soil was a deep-mouldy, fertile forest soil.

The upper horizons (*A*-horizon 0–10 cm, *B*-horizon 10–20 cm) consisted of a slightly loamy sand. The lower *C*-horizon alternated between clay and loamy sand.

The flora indicated a comparatively good access of light to the soil. The most common herbs were: *Anemone nemorosa* L., *Oxalis Acetosella* L., *Viola silvatica* Fr., *Urtica dioica* L., *Rubus idaeus* L. as well as *Deschampsia caespitosa* L. Beauv.

Tapping of the Exuding sap. The tapping of the exuded sap was made at 8–14 days' intervals by the following semi-sterile technique:

On each occasion two or three trees were selected. All roots were cut off at a distance from the base of the trunk of 10–30 cm. Thereafter the trunk was removed, and a pot-shaped hole dug in the ground about 1 m in diameter and 50 cm deep where the tree had

been standing. In this hole the severed roots were present, the free root length being about 20 cm. The roots which were found suitable for tapping were carefully washed in 2% sublimate over a distance of 15 cm from the surface of the cut. This part of the root was then cut off clean with a sterilized knife. Side roots, on the clean washed part of the root, if any, were likewise cut off with a sterilized knife. Immediately afterwards, a sterilized *v*-shaped glass tube was placed over the root by means of sterilized non wetting cotton wool for the collection of the exuding sap (see fig. 1). In order to prevent the

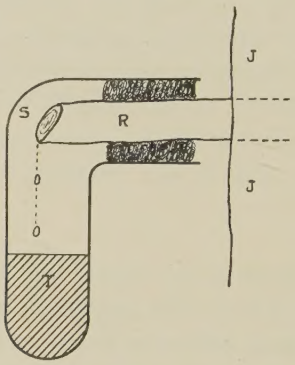

Fig. 1. Schematic drawing showing how the root tapping took place. *R* — root, *S* — surface of cut, *V* — non wetting cotton wool, *T* — exuding sap, *J* — soil.

development of micro-organisms in the latter, three drops of 15% sulphuric acid had previously been added to the tube, with the result that the pH of the sap gathered, even in the case of maximal tapping quantities was kept below 2.

The tapping time varied, for the different trees, between 12 and 36 hours.

The Chemical Analyses. When the tapping was finished, the tubes were removed from the roots, corked with sterilized, non wetting cotton wool plugs and immediately sent to the laboratory in the State Afforestation Research District. Here the exudate was analysed for the content of:

1. Phosphate,
2. Potassium,
3. Calcium,

4. Ammonia,
5. Organic nitrogen + ammonia,
6. Nitrate.

The analyses were carried out by Vagn Romose, Ph. D. Often the quantity of exudate was so small that it was only possible to carry out the determination of two or three of the above nutritive elements.

The analyses were made according to the following methods:

Phosphate was determined colorimetrically according to the Denigès–Atkins method cf. Denigès 1920 and 1921 [6]) and Atkins 1923/25 [2]).

Potassium was determined according to the most general practice: The potassium ion was precipitated as potassium-sodium-cobalti-nitrite. After washing, the precipitate was filtered off and determined by titration with permanganate. The analyses were carried out in conformity with the special technique worked out by Damsgaard–Sørensen, 1941.

Calcium was precipitated in an acetic solution with ammonium oxalate. The precipitate was washed out by means of a microfilter stick after which it was titrated against permanganate. The analyses were carried out according to the method given in Pincussen: Mikromethodik, p. 175, Leipzig 1930 [10]). Only a few calcium determinations were made.

Ammonia was determined by distilling samples of the sap, usually 10–15 cc, after addition of c. 2 g magnesia. The same distilling apparatus was used as for the „Mikrokjeldahl".

Ammonia + organically bound nitrogen were determined according to the method described by A. C. Andersen and Norman Jensen [1]) in 1931. In this test N 100 hydrochloric acid was used with iodometric acid titration. The determinations were made on quantities of sap of 2.5–5 cc.

Nitrate was determined according to the method worked out by Blom and Treschow (1928) [4]) and later improved by Treschow and Gabrielsen (1933) [11]). For each analysis 5 cc of sap was generally used. Before titration small quantities of silver sulphate were added in order to precipitate out chloride ions, (if any). With the quantity of sap used, the nitrate was, as often as not, below the limit of determination which is indicated in Table I by: 0-trace. At the same time it should be mentioned that the colorimetric analysis was not made using nitroxylenol as the standard solution. Each time sap analyses were made, a solution of potassium nitrate of a suitable and known nitrate content was analysed simultaneously.

Determination of the Root Depth. After the root tapping was completed, the roots were dug up, and the extension of each root in the vertical direction was measured. At the same time it was noted

TABLE I

Results of the Analyses

1 Date	2 Soil Moisture 0–10 cm %	2 Soil Moisture 20–30 cm %	3 Tree No.	4 Root No.	5 Root depth cm	6 Phosphate mg/l	7 Potassium mg/l	8 Calcium mg/l	9 Ammonia mg/l	10 Org. Nitrogen + Ammonia mg/l	11 Nitrate mg/l	12 Tapping Quantity cc
17/5	—	—	1	6	10-20	100	118 118 118	—	—	—	—	45
				8	20–40	97	70 72 71	—	—	—	0-trace	17
			2	11	0–10	97	228 228 228	—	—	—	0-trace	17
				12	10–20	86	193 200 197	—	—	—	0-trace	10
				10	20–40	128	88 83 86	—	—	—	0-trace	68
27/5	24.8	18.6	4	18	0–10	95	184 186 185	—	—	—	0-trace	48
				19	20–40	74	103 110 107	—	—	—	0-trace	41
				15	20–40	95	122 132 127	—	—	—	0-trace	31
				16	>40	80	110 110 110	—	—	—	0-trace	48
			5	21	0–10	105	161 164 163	—	—	190	—	20.5
				23	0–10	91	149 151 150	74	—	141	—	25.5
				25	0–10	76	172 176 174	95 116 106	—	123 123 123	0-trace	49.5
				28	10–20	50	133 133 133	64 66 65	—	118 117 118	0-trace	45.5
				29	10–20	52	131 132 132	82 84 83	—	105	0-trace	37.5
				27	20–40	—	120 120 120	—	—	—	—	5.0
				24	20–40	70	115 109 112	60	—	122 119 121	—	27.0
				26	>40	48	94 93 94	45 43 44	—	100 100 100	0-trace	44.5

				52	0–10	50	—	—	—	77 / 77	77	—	11.0
				33	0–10	63	73	—	—	50 / 44	47	—	15.0
				30	10–20	64	56	—	—	—		—	10.0
				34	20–40	85	—	—	—	—		—	2.5
			7	37	0–10	57	—	—	—		26	—	3.0
				35	0–10	43	48	—	—	82 / 73	78	0-trace	21.5
				40	10–20	90	94	—	0-trace	53 / 53	53	—	13.0
				41	10–20	69	44 / 44	44	—	—	52	0-trace	25.5
				44	10–20	74	—	—	—	57 / 53	55	0-trace	10.0
				38	20–40	41	61	—	—	38 / 30	34	—	14.5
				43	20–40	76	64	—	—	30 / 24	27	—	13.0
				42	>40	29	39	—	—	30 / 35	33	—	14.5
19/6	9.6	12.3	8	48	0–10	30	—	—	—		15	—	3.0
				47	20–40	38	—	—	—	11 / 11	11	0-trace	10.5
				46	20–40	34	—	—	0-trace	10 / 10	10	0-trace	14.0
			9	49	0–10	42	—	—	0-trace	11 / 9	10	0-trace	15.0
				50	0–10	42	—	—	—	13 / 5	?	0-trace	11.0
				51	10–20	50	—	—	—		16	—	3.0
28/6	27.4	14.9	10	52	0–10	50	64	—	0-trace	79 / 79	79	0-trace	45.5
				54	20–40	35	c.39	—	—	31 / 28	30	—	8.0
				53	>40	32	34	—	—	31 / 30	31	0-trace	27.0
			11	56	10–20	98	61 / 61	61	—	—	32 / 37	35	—	16.5
				57	>40	88	—	—	—		c. 8	—	2.5
			13	61	0–10	82	—	—	—	—		—	1.0
				62	0–10	143	—	—	—	22		—	5.0
				64	0–10	112	—	—	—	—		—	8.0
				63	10–20	64	—	—	—	—		—	1.5

TABLE II

Mean Content of Phosphate, Potassium, and Org. Nitrogen + Ammonia in the exuded sap from the Roots at various Depths. In ( ) is stated the number of roots

Layer-Depth cm	Date 17/5	27/5	7/6	19/6	28/6	Average
Soil Moisture %						
0–10	(25)	24.8	13.6	9.6	27.4	
20–30	(20)	18.4	12.9	12.3	14.9	
Phosphate mg/l						
	(1)	(4)	(5)	(3)	(4)	(17)
0–10	*97*	*92*	*58*	*38*	*97*	*74*
10–20	(2)	(2)	(4)	(1)	(2)	(11)
	93	*51*	*74*	*50*	*81*	*72*
20–40	(2)	(3)	(3)	(2)	(1)	(11)
	113	*80*	*67*	*36*	*35*	*70*
> 40	—	(2)	(1)	—	(2)	(5)
	—	*64*	*29*	—	*60*	*55*
Potassium mg/l						
	(1)	(4)	(3)	—	(1)	(9)
0–10	*228*	*168*	*68*	—	*64*	*130*
10–20	(2)	(2)	(3)	—	(1)	(8)
	158	*133*	*64*	—	*61*	*104*
20–40	(2)	(4)	(2)	—	(1)	(9)
	79	*117*	*62*	—	*c.39*	*88*
> 40	—	(2)	(1)	—	(1)	(4)
	—	*102*	*39*	—	*34*	*(69)*
Org. Nitrogen + Ammonia mg/l						
0–10	—	(3)	(5)	(2)	(2)	(12)
	—	*151*	*65*	*12*	*50*	*82*
10–20	—	(2)	(3)	(1)	(1)	(7)
	—	*112*	*53*	*16*	*35*	*62*
20–40	—	(1)	(2)	(2)	(1)	(6)
	—	*121*	*30*	*11*	*30*	*39*
> 40	—	(1)	(1)	—	(2)	(4)
	—	*100*	*33*	—	*20*	*43*

at what depth the main proportion of its root ends were to be found, it being assumed that it had absorbed its main nourishment from this layer. In Table I the depth of the layers, — to make the survey easier — have been stated in intervals of 0–10 cm, 10–20 cm, 20–40 cm, and 40 cm measured from the surface of the ground.

Results. The results of the analyses are given in Tables I and II.

The moisture content of the soil recorded in the tables was found in the following way: At the beginning of the tapping, samples of soil were taken from the depths 0 10 and 10 20 cm. The samples were air-dried to a constant weight at 20°C. The moisture content is given as the loss in weight of the soil in percentage of airdry weight.

The results of analyses of about 30 roots extending both in the uppermost and the deeper layers of the soil have not been included in the tables.

The following deductions can be made from the figures, though of course with a certain reserve, on account of the relatively small amount of material and the great variability:

1. When the soil had a relatively large moisture content the roots in the uppermost layers (the *A*-horizon) absorbed relatively larger quantities of potassium, (calcium) and nitrogen than did the roots in the deeper layers. The explanation must be sought in the fact that the uppermost layers are richest in nutrients, when there is sufficient moisture, as it is here the decomposition of the organic residual products takes place.

2. When the soil was badly dried up, as during the drought in June, the roots at 10 20 cm depth absorbed relatively larger quantities of nitrogen than the roots at 0–10 cm depth.

The explanation must no doubt be sought in a more strongly-reduced biological activity in the uppermost layer which was hardest hit by the drought.

3. The roots absorbed relatively larger amounts of the required nutrients during wet than during dry periods. The explanation must as above be sought in reduced biological activity during the drought.

4. At the same moisture content of the soil, the roots absorbed relatively larger quantities of potassium and nitrogen in May than in June. The explanation is presumably, that the content of these nutrients in the soil is, among other things, as shown by experience,

reduced during the period of growth independent of temperature and moisture status, cf. i. a. Lundegårdh (1925) [9]) and Honcamp (1931) [8]).

5. In contrast to potassium, (calcium) and nitrogen, phosphate was absorbed in relatively the same quantities by the roots in all layers; and at the same moisture content the roots absorbed relatively the same quantities in May as in June. It is difficult to give any satisfactory explanation of this exceptional condition. Possibly it is due to the fact that the quantity of phosphate was not so dependent on biological activity as was the quantity of the other nutrients. Alternatively the reason may be that phosphate was present as more readily soluble compounds.

It is a pleasure for me to tender my sincere thanks to the *Carlsbergfondet* which enabled me to carry out the investigations, and to Vagn Romose, Ph.D. for his careful laboratory work.

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PHYSIOLOGICAL EFFECT OF MINOR ELEMENTS IN CHILEAN NITRATE

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1. *Purpose and plan of the researches.* From previous investigations (1) the following conclusions have been drawn: in pot cultures, Chilean nitrate gives better results than other nitrates, this being primarily due to the boron content of the Chilean nitrate and its absence in the other fertilizers. Indeed, the small quantity of boron present in Chilean nitrate is sufficient to prevent heartrot of beets grown in pot cultures.

Observations, made during the vegetative period, however, suggested that, apart from the boron, the superiority of the Chilean nitrate was associated with other factors. As this superiority appeared chiefly at high rates of fertilizer applications, (which were much larger than those used in practice), this was attributed to other minor elements present in the nitrate.

In order to test this hypothesis, several series of investigations with pot cultures were carried out in 1938 to show the effect of minor elements other than boron.

The following plan gives a general outline of the experimental technique.

Series A was destined to show the specific effect of Chilean nitrate as compared with synthetic nitrate mainly at high rates of application (144 pots).

Series B and C were carried out with sand used previously and which therefore contained certain minor elements left behind by former dressings. Moreover the plants were watered with ordinary tap water the analysis of which revealed insignificant traces of boron but appreciable quantities of other minor elements (72 pots).

Series D was carried out with Hoagland's A–Z solution containing most of the available minor elements, including boron. To

show the specific effect of these elements, boron excepted, a series was added in which this latter element was absent (36 pots).

Series E compared Chilean nitrate with other commercial nitrogen fertilizers at equivalent nitrogen levels (72 pots).

Series F was carried out with a Campine sand on which heart-rot appears regularly. Watering was carried out with ordinary water in order to find out whether the minor elements in this water (almost free from boron) could prevent heartrot (144 pots).

As a basic dressing in all these experiments the following salts were applied per pot: 1.0 g of $MgSO_4$, 1.0 g of P_2O_5 as NaH_2PO_4, 0.8 g of K_2SO_4, 0.2 g of $CaCl_2$, 1 drop of a 5 percent solution of $FeCl_3$.

2. *Experimental results. Series A. Comparison of Chilean nitrate at increasing rates of application with equivalent rates of synthetic nitrate.* Sugar beet „Hilleshög 100/34" variety, distilled water, washed sand (see fig. 1).

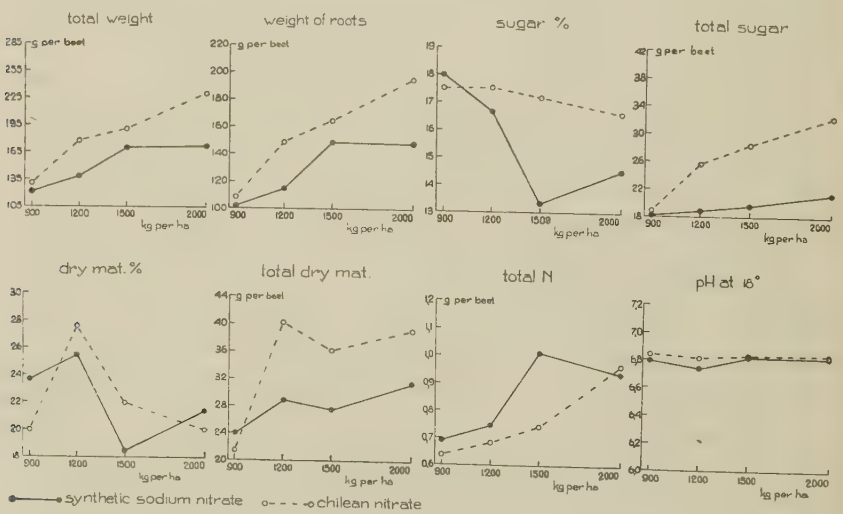

Fig. 1. Series A. Experiments with pot cultures. Comparison between Chilean nitrate and synthetic sodium nitrate.

It has been shown previously that Chilean nitrate was superior to synthetic sodium nitrate chiefly at the high rates of application. The yield curves for the natural nitrate showed a constant rise up to the rate of 1200 kg per ha, whilst those for the synthetic nitrate were flatter. The rates were increased to 2000 kg per ha in order to

find the point at which the curve for the Chilean nitrate began to fall off.

Total weight of the beets. At a rate of 2000 kg of nitrate per ha, the effect of the Chilean and synthetic nitrate is quite different, whereas they have much the same effect at the lower rate of 900 kg per ha.

Total weight of the roots. Similar results as for the total weight of the beets. The favourable influence of the Chilean nitrate appears not only on the roots but also on the whole plant.

The pH was much the same for the two nitrates at all rates The different effect was therefore probably not due to an eventual immobilization of the boron, which is this case should be chiefly influenced by the reaction, the aqueous systems having been the same. It is known that the immobilization of boron in soil is chiefly dependent upon the reaction of the soil.

Total nitrogen of the beets. A difference between the two nitrates is shown. The assimilation of Chilean nitrate, although rather small at lower rates, increases steadily at heavier rates up to the value of 2000 kg per ha whilst the maximum amount of assimilation with the synthetic nitrate is about 1500 kg per ha. Heartrot of beet appears to disturb the nitrogen metabolism of the plant.

Sugar content. The percentage of sugar decreases appreciably with heavy doses of synthetic nitrate but remains fairly constant with the Chilean nitrate. This, however, is not true for the total sugar content. In this case the curve for Chilean nitrate is noticeably steeper than that for the synthetic nitrate.

Dry matter. The percentages of dry matter are much the same in the two series. The slope of the total dry matter curve, however, is steeper for the Chilean nitrate than for the synthetic nitrate.

Appearance of heartrot disease. The following table gives some idea of the percentage of heartrot-diseased plants in this experiment.

Fertilizer rates kg/ha	Chilean nitrate % heartrot	Synthetic nitrate % heartrot
900	5.2	26.6
1200	5.1	100.—
1500	5.1	100.—
2000	5.2	100.—

Conclusions drawn from the results of series A. 1. Chilean nitrate is clearly superior to synthetic nitrate up to the rate of 2000 kg. Even at this dose the optimum rate for total weight, total sugar and total dry matter is not reached.

2. Total weight of the beets, the weights of their roots, the N content, the sugar percentage as well as the dry matter content are superior in the pots fertilized with Chilean nitrate.

3. These differences are governed chiefly by the presence or absence of heartrot disease, which occurs very frequently in the series with synthetic nitrate whilst the plant with Chilean nitrate have hardly been affected.

Series B and C. Comparison between synthetic sodium nitrate and ammonium nitrate (fig. 2). The sand for these experiments had been used in previous experiments. Watering was carried out with ordinary tap water. The object of these experiments was to find out whether a resulting accumulation of minor elements could or could not prevent heartrot disease.

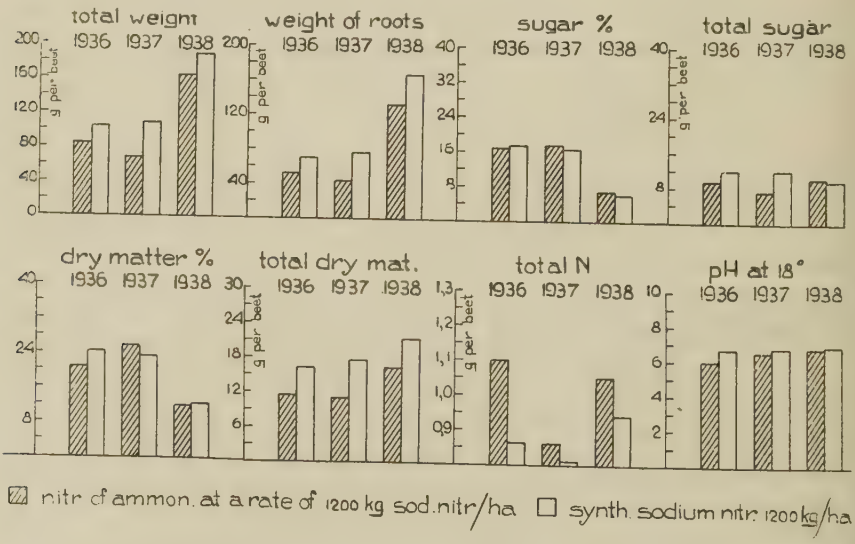

Fig. 2. Series B and C. Comparison of synthetic sodium nitrate with nitrate of ammonia on previous year's sand watered with tap water.

As the sand ages, both the total yield and vitality of the plants decrease in a similar fashion. This is the normal phenomenon of soil exhaustion whatever the cause may be: too highly concentrated mineral salts, lack of balance in microbial activity etc.

This ageing phenomenon acquires special importance owing to the fact that whilst the presence of boron prevented heartrot disease in the old sand the yield is still inferior to that in the new sand, where the disease appeared in 26% of the cases. The percentage of sugar, however, is notably lower in this sand. It is to be noted that the reaction remained sensibly unchanged during 3 ensuing years.

Series D: Hoagland's A-Z solution with and without boron. Total weight of the beets and weight of the roots (fig. 3). It can be stated that whilst the total crop yield and weight of the roots obtained with the A-Z solution without boron were somewhat inferior to those with the complete A–Z solution, this crop was appreciably superior to the one treated with synthetic nitrate.

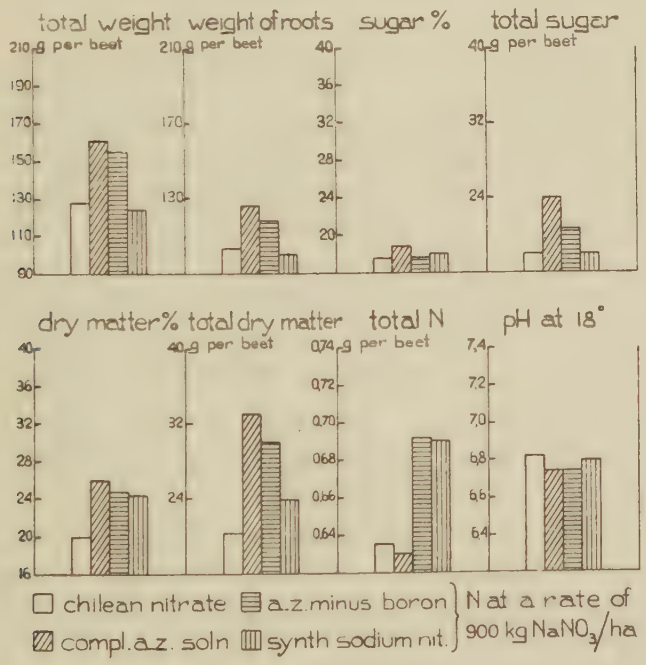

Fig. 3. Series D. Hoagland's A–Z solution with and without boron.

The analyses have been made only on healthy beets. Furthermore heartrot disease in the series with A–Z solution, minus the boron (25%) was much the same as that of the series with synthetic sodium nitrate (26.6%).

Chilean nitrate, under the same conditions, has given results appreciably inferior to those with the A–Z solution. Moreover it is barely superior to the synthetic nitrate, the rate of 900 kg having been too low to bring the minor elements up to a satisfactory physiological level.

Sugar percentage and total sugar. The sugar % in the series without boron is somewhat inferior to that of the series with boron.

The total sugar is highest in the complete A–Z series and lowest in the series with synthetic and Chilean nitrate. It is intermediate between the two in the series A–Z minus boron.

Dry matter. Same as for the sugar and the total weight.

Total nitrogen. Highest in A–Z series without boron and with synthetic sodium nitrate, considerably lower with Chilean nitrate and with a complete A–Z-solution.

Reaction. Absolutely the same in the 4 series. It cannot therefore in any way have favoured the appearance of heartrot.

Heartrot. 0 with the complete A–Z solution plus boron. 25% with the A–Z solution minus boron.

Conclusions. The results, obtained with Hoagland's A–Z solution have been compared with those obtained in the corresponding Chilean nitrate series, at the rate of 900 kg using the sand of 1938. At this dose, the Chilean nitrate and synthetic nitrate are of much the same value, as can be seen in the diagrams of series A, except that the % of plants injured by heartrot is lower in the Chilean nitrate series.

With the A–Z solution minus boron, 25% of the plants were diseased. The healthy plants, however, were of an appreciably higher productivity.

It is clear from the above results that the boron contents are decisive in the appearance of heartrot disease, and that, apart from the boron, the other minor elements do have some influence on the general physiology of the beet.

That these minor elements are not present in sufficient quantities in the Chilean nitrate is shown by the productivity of the series A–Z minus boron, which is certainly superior to that of the corresponding series of Chilean nitrate applied at the rate of 900 kg per ha. To obtain the effect of the minor elements in the Chilean nitrate, we should have to increase the rates of application. It has already been

shown that this specific effect can be produced at the rate of 2000 kg per ha.

Series E. Fodder beets with Chilean nitrate, synthetic nitrate, nitrate of ammonia and nitrate of lime (at a rate of 1200 kg Chilean nitrate per ha).

Washed sand. Watering with distilled water.

Total weight of the beets and weight of the roots (fig. 4).

The results are ranged in decreasing order as follows: Chilean nitrate, synthetic sodium nitrate, nitrate of lime, nitrate of ammonia. They agree with those of previous experiments on sugar beets.

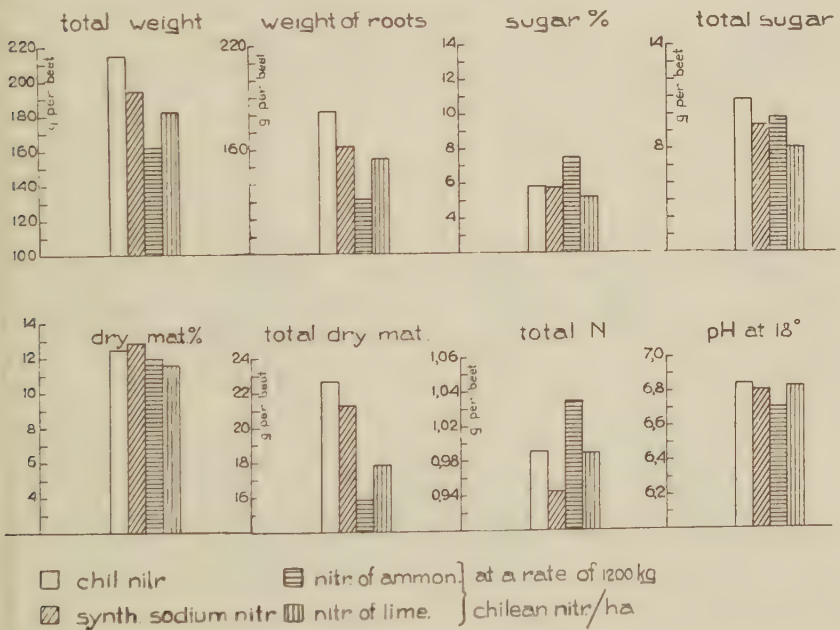

Fig. 4. Series E. Fodder beets, sand 1938.

% Sugar and total sugar. It is interesting to note the high sugar content due to the nitrate of ammonia.

Dry matter. The percentage of dry matter is much the same in the 4 series. The total dry matter is highest with the Chilean nitrate.

Total nitrogen. Ammonium nitrogen not only gave rise

to the highest sugar content but also the highest value for total N. The depression of the yield with this fertilizer cannot be caused by a lack of assimilable nitrogen, but may be due to a deficiency of minor elements, such as the results of the other series appear to suggest. The relative accumulation of nitrogen with the nitrate of ammonia seems to indicate that the utilisation coefficient of this fertilizer for the beet is not as high as that of the simple nitrates.

Beet is a "carbohydrate" plant, the importance of the yield being primarily determined by the production of carbohydrates. If the limit is reached, a surplus of nitrogen fertilizer is no longer used to produce more carbohydrates but to accumulate protein matter. This relative accumulation of organic nitrogen is profitable for ,,protein" plants used as concentrated food for cattle, but not for those plants which have to produce sugar and starch.

The reaction: is much the same in the 4 series; it is somewhat lower with ammonium nitrate than with the other nitrates which are physiologically alkaline.

Heartrot disease. Absent in the Chilean nitrate series, it appeared sporadically in the other series. These results confirm those obtained with sugar beets in the previous years.

Conclusions: From the above it appears that Chilean nitrate has also a favourable effect on the physiological development of fodder beets. This influence is specific to this fertilizer alone and absent with the others. The effect, however, is not only due to the presence of boron, since all these results refer to healthy beets.

Series F. Experiments with fodder beet on a Campine soil where heartrot disease occurs locally and regularly. The object of these experiments was to find out whether the appearance of heartrot disease was caused by absence of boron or by other factors. The field in question was divided into 2 parts, one part where the disease appeared constantly, the other part where it was rather rare.

In the present experiment the soil of the 2 parts was mixed in order to see whether the relative boron richness of the healthy part would be sufficient to "cure" the diseased part.

In the pot cultures no plants were affected by the disease. Obviously the low boron content of the healthy part of the field was high enough to ,,cure" the sick part. In this experiment watering was carried out with ordinary tap water, bringing minor elements other than boron into the soil.

In these as in the other experiments the particularly favourable effect of the Chilean nitrate appears clearly and chiefly at the heavy rates (fig. 5). From this point of view, it is very interesting to compare the series F with the series E in sand. The general results are much the same. Not only in washed sand but also on certain soils as well, is the superiority of the Chilean nitrate evident in comparison with the other nitrogenous fertilizers.

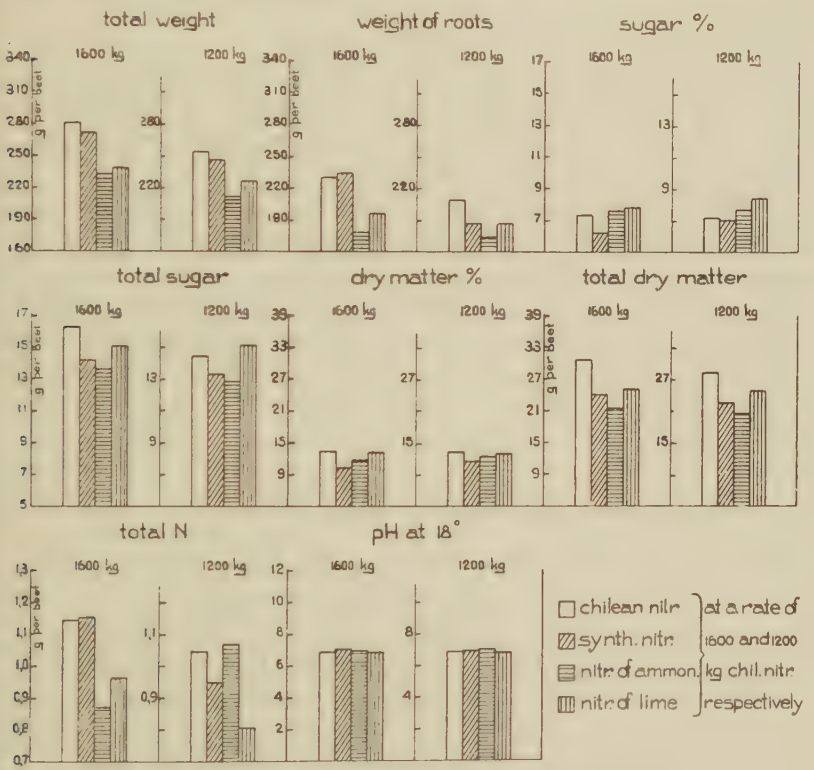

Fig. 5. Series F. Fodder beets on a Campine soil.

3. *General conclusions.* 1. Chilean nitrate has a specifically favourable effect as contrasted with other forms of nitrogen fertilizers. This effect has been observed during a period of 3 consecutive years. It appears in fodder beets and also in sugar beets and is chiefly perceptible at high rates which are normally depressing with the other fertilizers.

2. This specific action is partially due to the prevention of

heartrot disease. Besides the boron, contained in Chilean nitrate, other elements probably play a part. The following facts tend to conform this hypothesis:

a. The series with a complete Hoagland's A–Z solution yields a crop appreciably higher than the corresponding series with Chilean nitrate, because the latter has a far lower minor element content than the former. Moreover the series with the A–Z solution, without boron, shows a greater productivity and at the same time a higher heartrot disease percentage (25%).

Thus two facts have to be considered: the general growth of the plant, being under the influence of minor elements as a whole, including boron, and the appearance of the heartrot disease depending chiefly on the presence or absence of the boron.

b. All the above determinations (crop figures and analyses) have been carried out on healthy beets. Even in these beets which contain enough boron to prevent heartrot disease, Chilean nitrate is still greatly superior to synthetic nitrate in nearly all the series. This indicates that the superiority must be due to other elements present in the Chilean nitrate.

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THE TRANSFORMATION OF CALCIUM CYANAMIDE IN THE SOIL*)

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Introduction. About 1900 Gerlach and Wagner [11]) found that Ca-cyanamide could be used as a nitrogen fertilizer. From that time a great number of papers dealing with the decomposition of Ca-cyanamide in the soil were published. It was shown that cyanamide, as such, could not be used by the plant as a source of nitrogen. So it has to be changed to other nitrogen compounds before it can be assimilated by the plant.

Immendorff [15]) supposed that the transformation of Ca-cyanamide was brought about by a chemical process following the equation:

$$CN_2Ca + CO_2 + 4H_2O \rightarrow (NH_4)_2CO_3 + CaCO_3$$

Behrens [3]), however, suggested that the ammonification of $CaCN_2$ (calcium cyanamide) was the result of a biological process, the same as that for the conversion of urea into ammonium carbonate. Lochnis [19]), [20]) and coworkers also stated that Ca-cyanamide in soil was broken down into ammonium compounds by the action of micro-organisms. Many workers affirmed this statement [2]), [23]).

Ulpiani [29]), [30]), [31]) came to the conclusion that Ca-cyanamide in soil is converted by physico-chemical means, viz. 1. A quickly accomplished hydrolysis from cyanamide into urea.

2. A polymerisation, slowly brought about, from cyanamide into dicyanodiamide.

In both processes the micro-organisms of the soil do not play any

*) This paper deals with a part of the thesis by the author, entitled: „Over de afbraak van Ca-cyanamide in den grond", published in 1946 (Wageningen).

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part. These speculations caused a controversy between Ulpiani and Loehnis. Loehnis [21]) eventually showed the correctness of the Ulpiani theory, as far as the hydrolysis of cyanamide into urea was concerned. Kappen [15]), [16]) also gave further proof for this theory about the physico-chemical hydrolysis of cyanamide into urea, being the first stage of the transformation of cyanamide in soil. It turned out to be more difficult, however, to prove the second part of Ulpiani's theory, viz. the polymerisation of cyanamide into dicyanodiamide.

Stutzer and Reis [28]) came to the same conclusions as Ulpiani and Kappen concerning the hydrolysis of cyanamide, although they did not wholly exclude the co-operation of the micro-organisms in the process of urea formation from cyanamide.

Thus, since 1913 it has been generally assumed that Ca-cyanamide in soil is converted into ammonia along the following stages:

1. Ca-cyanamide hydrolyses into cyanamide and Ca-hydroxide.

$$CaCN_2 + 2H_2O \rightarrow H_2CN_2 + Ca(OH)_2.$$

2. Cyanamide is subsequently hydrolysed into urea by physico-chemical means:

$$H_2CN_2 + H_2O \rightarrow CO(NH_2)_2$$

3. Urea is finally converted into ammonium carbonate by micro-organisms.

$$CO(NH_2)_2 + H_2O \rightarrow (NH_4)_2CO_3.$$

Although this conversion was shown in our investigations to be correct, it was clear that the problems around this scheme are far from solved. Most of the named investigators made their experiments with aqueous solutions instead of soil, while the concentrations of cyanamide used in their experiments were too high (from 10 to 100 times as high as the concentration in the soil after manuring with Ca-cyanamide under agricultural conditions). Moreover the circumstances under which the transformation experiments have been made (high temperatures, boiling of solutions containing cyanamide with strong acids or alkalis) were such that the application of their results to the processes in soil is liable to error. So the experiments and theories about Ca-cyanamide up to 1914 must be accepted with reservation. The experiments of Cowie [6]), [7]) and of Jacob Allison and Braham [14]) are more in accordance with normal

agricultural conditions. Cowie affirmed the statements of Ulpiani about the formation of urea from cyanamide, while Jacob c.s. studied more in detail the nitrification of cyanamide in the soil. Meanwhile, Mazé and Lemoigne [22]) stated that certain micro-organisms were able to hydrolyse cyanamide to urea on condition that the cyanamide concentration in the culture solutions was low enough to cause no injury to the test organisms.

In 1938, Schmalfusz [27]) concluded that the prevailing opinions about the transformation of cyanamide into urea in the soil lacked proper fundamental proof. He confirmed the opinion of Mazé and Lemoigne about the biological urea formation from cyanamide. In the experiments of Schmalfusz, *Aspergillus niger* was shown to be very active in this respect. He came to the conclusion that the bacteria and fungi in the soil play the dominant part in the conversion of cyanamide into urea and that the physico-chemical hydrolysis of cyanamide was of minor importance. It will be shown in this paper that these ideas of Schmalfusz are not exact.

Contrary to his last theory, Rotini [26]) defended once again the physico-chemical standpoint of Ulpiani and Kappen. Thus, two conflicting views exist as to the mode by which urea originates from cyanamide viz.

1. The soil induces by means of physico-chemical action the conversion of cyanamide into urea. In this conversion micro-organisms either play no part or only a minor one.

2. Urea is formed out of cyanamide by micro-organisms. The physico-chemical hydrolysis of cyanamide is, when compared with the biochemical one, of minor importance.

In this paper some experiments are described in which this issue is clarified. The question concerning the formation of dicyanodiamide will be left undecided, because it was not possible to determine small amounts of this compound in the soil. Indirectly it was shown that in the decomposition of cyanamide under optimal conditions the formation of dicyanodiamide needs not to be apprehended.

Experimental methods. For the present experiments it was considered important to use such concentrations of cyanamide in soil which will originate after an application to the soil of a normal quantity of Ca-cyanamide. Therefore the concentration of cyanamide used in our experiments was based on a manuring with Ca-

cyanamide corresponding to 60–90 kg of nitrogen per ha (30–45 lb of nitrogen per acre).

To obtain comparable results it was decided to add to the soil the cyanamide in the form of an aqueous solution. This cyanamide solution was obtained by shaking 5 g of Ca-cyanamide during one hour with a given quantity of water. After this, the excess of calcium hydroxide was removed by passing through the mixture CO_2-gas until pH 7 was reached. The cyanamide extracts contained nitrogen in the form of cyanamide and a small quantity in the form of urea. They contained no nitrogen in the form of ammonia, while probably a small quantity of dicyanodiamide was present. Extracts containing from 10 to 15 mg of N in the form of cyanamide were used.

For each conversion experiment several portions of 100 g of soil in glass beakers were prepared. After the cyanamide extract was added, the soil was moistened to 50–60% of its water capacity. Then the beakers were incubated at constant temperature. From time to time some of the portions were percolated with a N/2 solution of KNO_3 or K_2SO_4. In the percolates the reaction products of the cyanamide conversion were determined.

Analytical methods

Total N. The method of Kjeldahl-Lauro [18]) was used.

Cyanamide. A method, based on the principles given by Caro [5]) was used. The cyanamide was separated from the soil percolates as the precipitate with silver nitrate after the solution had been made alkaline with dilute ammonia. The precipitate was collected on a filter paper and, after careful washing, was digested after the method described for total N. After a simple procedure the cyanamide content can be calculated.

Urea. Urea in soil filtrates can be determined by different methods viz. the *direct* method of Fosse [9]), Allan and Luck [1]) with Xanthydrol, and the *indirect* method with urease after Cowie [6]), [7]), Schmalfusz [27]), Fox and Geldard [10]). The indirect method was used in these experiments. The methods of Schmalfusz (urea is changed with urease into ammonia, the ammonia being subsequently distilled "in vacuo" at 45–50°C) and that of Fox and Geldard (here, urea is titrated, after conversion into ammonia with urease, with 0,1 or 0,01 N hydrochloric acid. As indicator a mixture of bromthymolblue and bromcresolpurple 1 : 1 was used). The last method is only useful in non-buffering solutions.

Ammonia. The filtrates were pipetted into flasks and distilled, after addition of MgO, at 100°C or, if necessary, at 45–50°C "in vacuo".

Nitrate. The method after Blom and Treschow[4]) was used. This method is based on the nitrating (by HNO_2 and HNO_3) of meta-2-4-xylenol in a solution made acid with sulphuric acid (80–85%). The brownish-yellow coloured nitro-xylenol is distilled into a solution of N/5 NaOH. The intensity of the colour of the yellow distillate (sodium-salt of nitro-xylenol) is estimated in a colorimeter by comparison with known nitrate concentrations.

Dicyanodiamide. As stated previously no method was found for estimating dicyanodiamide.

Most of the following experiments were made with a sandy garden soil (L 32) from the experimental-fields of the Microbiological Laboratory of the Agricultural College, Wageningen. This soil has the following characteristics:

water capacity	32 %
organic matter	4 %
silt fraction 16 2μ	4 %
silt fraction $< 2\mu$	5 %
$pH_{(H_2O)}$	5.9
$pH_{(KCl)}$	4.9

The conversion of cyanamide in soil unchanged by heat or antiseptics. As a starting-point for further investigation we analysed the conversion of cyanamide in the soil L 32. The beakers containing soil L 32 supplied with cyanamide extract were incubated at 23°C. From time to time analyses for cyanamide, urea and ammonia were made. The results of this experiment (presented in Table I and Figure 1) show that the cyanamide is gradually disappearing with duration of experiment.

TABLE I

Transformation of cyanamide in soil L 32

Analyses after	Number of beakers analysed	mg N in 100 g of soil as		
		Cyanamide	Urea	Ammonia
0 hr	3	10.3	0.9	0.0
6 ,,	3	8.5	1.0	0.0
12 ,,	5	7.2	1.2	2.9
24 ,,	5	5.2	1.4	3.6
36 ,,	5	2.3	2.3	6.7
48 ,,	3	2.0	2.0	6.0
60 ,,	2	tr.	0.9	7.8
72 ,,	1	tr.	0.5	8.8
108 ,,	1	0.0	0.0	9.6

After 24 hr incubation 50% of the cyanamide supplied to the soil had been transformed; after 108 hr the cyanamide had wholly disappeared.

Urea only accumulated during a short period presumably in consequence of the activities of the microflora. The quantity of urea increased up to 36 hr of incubation. When the micro-organisms which decompose urea have fully been restored (the soil had been air-dried) ammonia gradually accumulated from its decomposition.

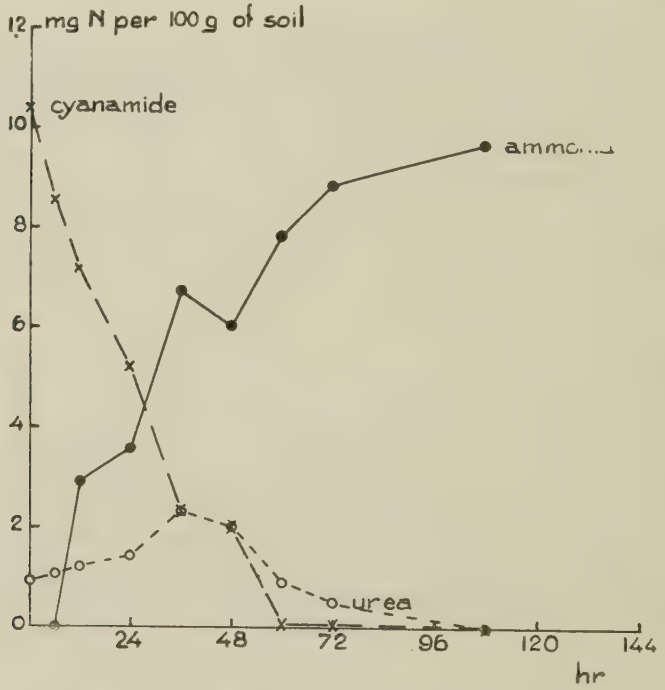

Fig. 1. Conversion of cyanamide in soil L 32.

It is obvious that Wienhues[3]) was incorrect when he stated: "in consequence of the extraordinary quick ammonification of urea, no urea formation in soil can be determined after a manuring with Ca-cyanamide".

The rate of disappearance of cyanamide estimated in the present experiment is in accordance with the results of Crowther and Richardson[5]). The speed with which the cyanamide in soil is transformed depends on the moisture content and on the temperature under which the conversion takes place.

The line that indicates the disappearance of cyanamide (see fig. 1) is in accordance with the formula of a monomolecular reaction. This indicates that the cyanamide is disappearing according to the equation:

$$H_2CN_2 + H_2O \rightarrow CO(NH_2)_2 \text{ *)}$$

As the polymerisation of cyanamide into dicyanodiamide is a bimolecular reaction there is no place for the formation of this compound under the conditions of the experiment just mentioned. P r a n k e [21] also states that "in good agricultural practice very little, if any, dicyanodiamide is formed in the soil". At the end of this paper some cultural conditions for good agricultural practice are stated.

Conversion of cyanamide in heated soil. In the previous experiment it was shown that in the soil urea is formed after an application of Ca-cyanamide. Now it was necessary to investigate which part of the soil is responsible for the transformation of cyanamide into urea. The micro-flora and the chemical activities of certain parts of the soil receive full consideration in this respect. In sterilising the soil, whether by heat or by chemical compounds, it is possible to separate these two main groups of activities one from another. In the sterilised soil the biochemical activity can be eliminated while the influence of the colloidal-chemical properties of the soil on the conversion process are studied.

Nevertheless it is very difficult to obtain completely sterile soil. W a k s m a n [22] showed that soil can be sterilised by heating it during 2 hr in an autoclave at approx. 115° C, or by steaming during 1 hr on 7 successive days. Heating the soil during 1 hr at 100° C will kill only certain groups of micro-organisms. The same holds for a treatment of the soil with certain antiseptics (toluene, $HgCl_2$, etc.).

For the purpose of the experiment it seemed necessary to use sterile soil. On the other hand it was necessary for the condition of the colloidal matter of the soil to remain practically undisturbed. A compromise between these two requirements was therefore adopted. In the preceding experiment it was shown that the cyanamide in the soil had disappeared within 100–110 hr incubation at 23° C. There-

*) This reaction acts like a monomolecular one, as the concentration of the H_2O-part is in an important excess when compared with the concentration of H_2CN_2.

fore a treatment which kept the soil in "biochemical rest" during 4–5 days was used.

The following treatments were compared:

a. Steaming the soil twice at 100°C during half an hour with an interval of 24 hr.

b. Heating the soil twice at 115°C during 20 min with an interval of 24 hr.

In both cases the soil was supplied with water before heating, since the micro-organisms are more easily killed in a wet soil than in a dry one.

To learn something about the conversion of cyanamide and the restoration of the micro-flora in these heated soils a quantity of cyanamide extract was added to the portions of soil. From time to time quantitative tests for cyanamide, urea and ammonia were made while the numbers of surviving micro-organisms growing on broth agar (pH 7.2) were also counted. Results of these experiments are summarised in Tables II and III and Fig. 2 and 3.

TABLE II

Conversion of cyanamide and plate counts of bacteria in soil heated at 100°C. (Temp. of incubation 23°C.)

Analyses after:	Numbers of bacteria per g of soil	mg N per 100 g of soil	
		Urea	Ammonia
0 hr *)	— †)	0.7	—
27 ,,	— †)	2.6	2.4
75 ,,	200 000 §)	4.3	2.4
100 ,,	5 000 000	5.0	3.0
148 ,,	500 000 000	3.9	4.7
264 ,,	—	1.8	8.7

*) immediately after the second heating.
†) counts are unreliable; numbers of colonies on plates were too small.
§) distinct change in character of micro-flora.

From the results of this experiment it is seen, that the soil, immediately after heating at 100°C contains a very small number of micro-organisms. During the first 27 hours the plate counts remain very low. After 27–75 hours incubation a very large increase of the number of bacteria occurs. It is remarkable that this increase includes only a small number of species.

The flora after 75 hours of incubation mainly consisted of representatives of the *Pseudomonas* group. It was shown (with controls on

malt agar) that the heating of the soil and subsequent addition of cyanamide excluded wholly the fungi and probably the spore-forming bacteria.

In other experiments it was shown that Ca-cyanamide, as such, has a selective action on the micro-flora of the soil. This is in accordance with the communications of other investigators [17], [3]). Nevertheless soil heated at 100 C is not long enough in "biochemical rest" to exclude the activity of micro-organisms in the process of urea formation from cyanamide. (See also fig. 2).

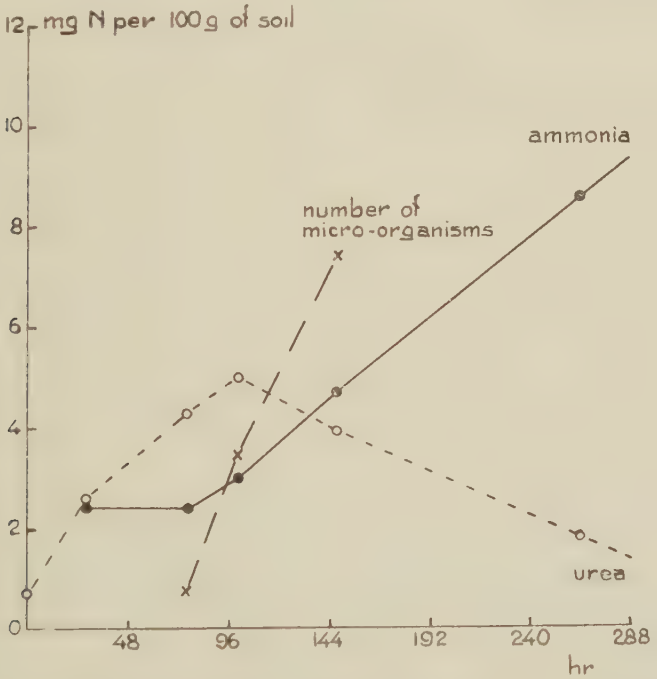

Fig. 2. Conversion of cyanamide in soil L 32, heated during one hour at 100°C. (Number of micro-organisms as 2 [log. (number of micro-organisms $.10^{-5}$)].

The soil, treated at 115° C gave results shown in Table III and Fig. 3.

In this case, no plate counts have been made as it was clearly shown that these counts could be replaced by the data given by the analyses of the ammonia content of the soil. If biological activity is suppressed there is no accumulation of ammonia. From the results of

TABLE III

Conversion of cyanamide in soil heated at 115°C. (Incubation temperature 23°C.)			
Analyses after:	mg N per 100 g of soil		
	Cyanamide	Urea	Ammonia
0 hr	14.5	0.8	2.9
12 ,,	10.9	4.0	2.8
24 ,,	8.8	—	—
36 ,,	6.8	7.5	2.9
48 ,,	6.0	8.1	2.8
96 ,,	3.3	10.0	3.4
144 ,,	1.0	11.2	3.4
264 ,,	0.0	11.6	3.1

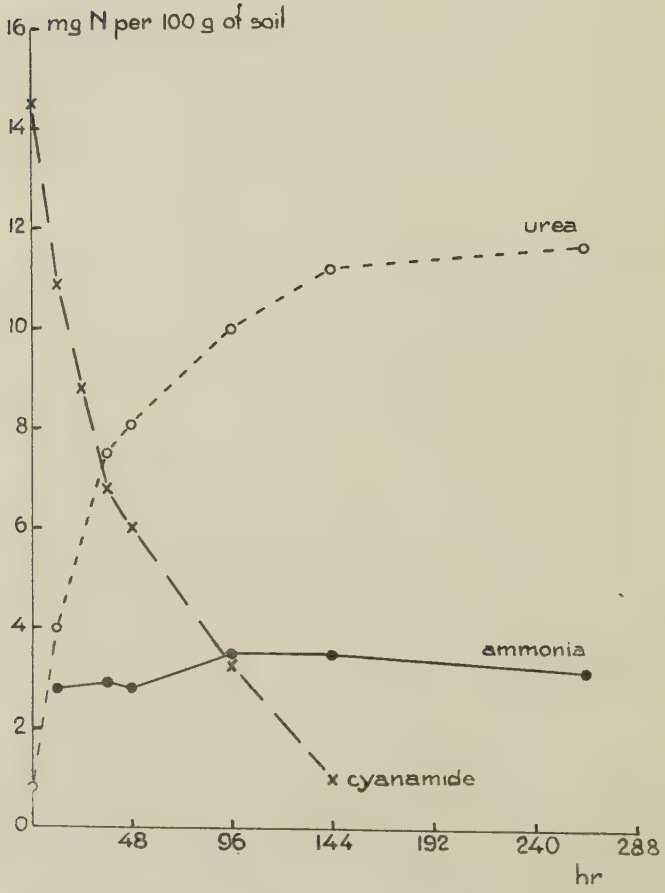

Fig. 3. Conversion of cyanamide in soil heated at 115°C.

this experiment it is concluded that the transformation of cyanamide into urea is induced by physico-chemical action. Micro-organisms are playing no part in this process. In living soil the activity of the micro flora prevents this chemical process being seen. It will be seen that 11.6 mg of urea were formed from the 14.5 mg of cyanamide initilaly

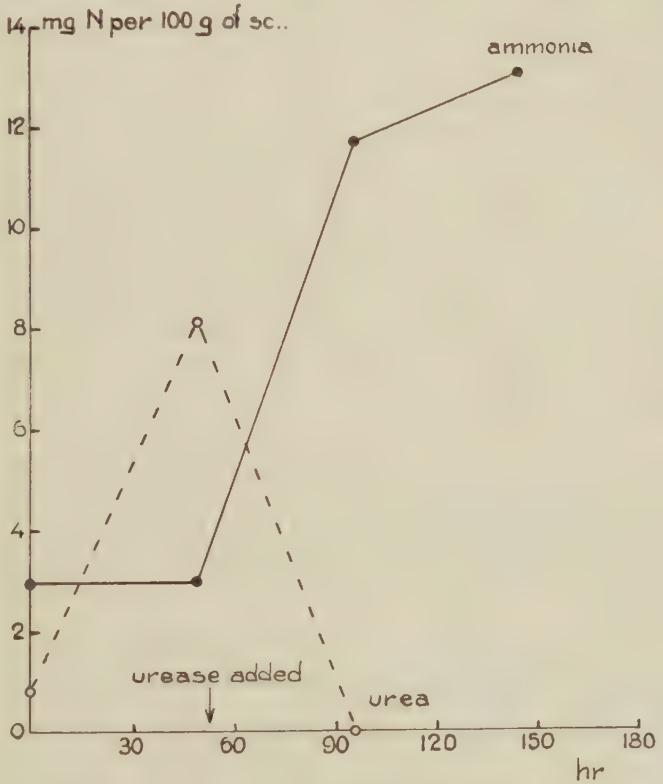

Fig. 4. Transformation of cyanamide in sterile soil supplied with urease.

present in the soil. Thus a loss in the nitrogen balance of nearly 3 mg was substained. Probably, this is the result of the formation of a small quantity of dicyanodiamide.

In other experiments, not recorded here, it was shown that the exclusion of the micro-flora causes abnormalities in the conversion process. It is suggested that this exclusion involves an equilibrium in the reaction: cyanamide $\rightleftarrows$ urea, by which a part of the cyanamide will be polymerised into dicyanodiamide. This may be of importance when the decomposition of cyanamide in normal soils is considered. Here the formation of dicyano-

diamide is unlikely for it is improbable that an accumulation of urea exists in the living soil.

The above experiments indicate that cyanamide in the soil is converted, first of all, into urea by some chemical means. In this process the micro-organisms play no part. After urea has been formed the micro-flora hydrolyses the urea into ammonium carbonate. The following experiment provided further evidents of this.

To several portions of soil, heated at 115°C, a cyanamide extract was added. After 48 hours of incubation at 23°C a sterilised solution of urease (an aqueous solution of Urease-Yonkers U.S.A.) was added to the soil under sterile conditions. From time to time analyses were made for cyanamide, urea and ammonia content. Fig. 4 shows that the urea content in the soil gradually increased until the moment at which the urease was added. From this moment onwards the urea quickly disappeared following conversion into ammonia. This experiment can be considered as an imitation of the transformation of cyanamide in the soil.

The action of micro-organisms on cyanamide. Although it seems clear that the hydrolysis of cyanamide in the soil occurs without the co-operation of micro-organisms, it is necessary to consider the way these organisms take part in the decomposition processes in "living" soil.

It is known that there are micro-organisms which hydrolyse cyanamide into urea .Moreover, it is well known that micro-organisms can utilise cyanamide as a source of nitrogen in composting processes. As appears from the data recorded in Table IV, the micro-organisms react differently when grown in a solution containing glucose and cyanamide.

TABLE IV

Assimilation of nitrogen by different species of bacteria

Cyanamide added to solution (mg N)	mg N assimilated by:					
	Bll. spec. after		*Bm. pyocyaneum* after		*Aspergillus niger* after	
	7 days	20 d	7 d	20 d	7 d	20 d
7.1	1.1	4,7	2.7	2.8	6.8	—
13.4	2.1	7.1	8.1	8.0	13.4	—
28.7	3.0	11.5	14.3	22.7	14.1	—

On testing the micro-organisms for the formation of urea in a solution containing glucose and cyanamide, *Aspergillus niger* was shown to be very active in this respect [27]). Schmalfusz suggested that *Aspergillus niger* possesses an enzyme or a catalysing agent of inorganic character which brings about the hydrolysis of cyanamide. This hydrolysing effect of *Aspergillus niger* is shown in Table V.

TABLE V

Transformation of cyanamide into urea by *Aspergillus niger* in a medium containing glucose and cyanamide

Analyses after	Total N in solution	pH of solution	mg N per 120 ml of solution			
			Cyanamide	Urea	Ammonia	Organic
0 days	28.4	8.3	25.8	2.0	—	—
5 ,,	—	4.9	—	13.3	3.0	—
7 ,,	25.1	4.1	7.3	16.5	2.9	1.9

As is shown, *Aspergillus niger* is able to transform the cyanamide into urea very rapidly. After 5 days incubation at 30°C, one half of the given cyanamide was hydrolysed.

Incidentally, the pH of the solution was fixed too high (pH: 8,3) Unexpectedly, this gave a clear insight in the hydrolysing power of the mass of hyphae of *Aspergillus niger* as the growth of the fungus after 5 days was not very abundant.

After the above experiment the mycelia were collected and washed with distilled water for two hours. The mass of hyphae was then transferred into sterile Erlenmeyer-flasks together with a quantity of a cyanamide extract (pH 4.5). Toluene was added to prevent contamination and the growth of the hyphae. From time to time the flasks were analysed for urea and ammonia.

The results are summarized in Table VI.

TABLE VI

The formation of urea from cyanamide by *Aspergillus niger*

Analyses after	mg N per 120 ml solution	
	Urea	Ammonia
20 hr	3.7	1.6
42 ,,	7.5	1.5
116 ,,	12.3	4.5
164 ,,	19.6	3.0

It is clearly shown that *Aspergillus niger* can quickly convert cyanamide into urea. The somewhat fluctuating data for ammonia are probably the result of an irregular autolysis of the hyphae. Now, for the second time, the mycelia of the fungus from the preceding experiments were collected and disintegrated in a ball-will with distilled water. The ground mass of hyphae was then filtered within six hours in a refrigerator. Both the filtrate and the ground mycelia were used in the following experiment. In this a solution of cyanamide (after the addition of toluene) was mixed with the sterilised filtrate or the ground mycelia.

The analyses are summarized in Table VII.

TABLE VII

The formation of urea and ammonia by the ground mycelium of *Aspergillus niger* and its aqueuos extract

Analyses after	N in mg per 130 ml solution								
	Flasks with filtrate of ground mycelium						Flasks with ground mycelium		
	Solution containing Cyanamide and Urea			Solution containing Cyanamide			Solution containing Cyanamide		
	Urea	Ammonia	pH	Urea	Ammonia	pH	Urea	Ammonia	pH
0 hr	17.4	1.1	6.9	0.8	1.0	6.9	0.8	—	6.9
64 ,,	46.2	1.2	6.9	34.5	1.0	6.9	8.7	15.4	7.5
88 ,,	41.3	1.1	6.7	—	1.3	6.8	11.3	29.8	8.5

From the results given in Table VII, it is seen that the extract of the ground mycelium of *Aspergillus niger* has the property of converting cyanamide into urea. The ground mycelium also has this property, although not to the same extent. After 64 hours it had formed 8 mg of urea, while the filtrate had hydrolysed 33.7 mg of cyanamide into urea. It is shown that the filtrate of the ground mycelium is not able to convert urea into ammonia. Therefore the enzyme which causes the hydrolysing of urea was not liberated in the grinding-process or it was inactivated after it had been separated from the living cell. From the foregoing results it is clear that the complex which causes the hydrolysis of cyanamide and the urea-splitting enzyme are not the same, since these two agents were seperated by the succesive grinding and filtering of the mycelium.

The experiments described suggest that there are micro-organisms in the soil which are able to convert cyanamide into urea. From the experiments with cyanamide in heated soil, however, it seems that the cyanamide will be converted at such a rate that the micro-orga-

nisms cannot take any part in it. Particularly since the microflora has to adapt itself to the fertilizing with Ca-cyanamide. This leads to the so-called "cyanamide-shock", which induces an initial decrease of the number of micro-organisms in the soil. Although this decrease will be soon followed by a large increase, the cyanamide will have disappeared when the micro-flora has adapted itself. Moreover, the soil L 32 did not contain *Aspergillus niger* (as shown by a test on malt agar) after it had been dressed with Ca-cyanamide. Therefore it is impossible that, in this soil, *Aspergillus niger* has caused the quick hydrolysis of cyanamide into urea.

As many bacteria in the soil develop well in a solution which contains glucose and cyanamide as a source of nitrogen, it may be readily conceived that these bacteria will also be able to hydrolyse cyanamide into urea. A large number of experiments shows, however, that the rate of the bacterial hydrolysis is small compared with the rate of hydrolysis in the soil by physico-chemical means. (See table VIII).

TABLE VIII

The formation of urea and ammonia from cyanamide by certain strains of bacteria isolated from a soil fertilized with Ca-cyanamide

Analyses after	mg N per 25 ml of solution							
	strain 1		strain 5		strain 8		strain 13	
	Urea	Ammonia	Urea	Ammonia	Urea	Ammonia	Urea	Ammonia
0 hr	0.37	0.0	0.37	0.0	0.37	0.0	0.37	0.0
6 ,,	0.25	0.13	0.21	0.18	0.22	0.16	0.22	0.17
12 ,,	0.24	0.16	0.31	0.16	0.27	0.21	0.29	0.17

Some remarks on the ammonification and nitrification of Ca-cyanamide. It has been shown that the activities of the micro-organisms in the process of transformation of Ca-cyanamide in the soil only begin after the formation of urea. The urea is, for the greater part, converted into ammonium carbonate, a small part being used by the micro-organisms themselves. The ammonifying organisms are but little influenced by the "cyanamide-shock" which originates in the soil immediately after a dressing with Ca-cyanamide. It was shown that a dressing with Ca-cyanamide, corresponding to an application of 30 kg N per ha, acts the same, in respect to the ammonification, as a manuring with urea which provided the same quantity of nitrogen. Under the same conditions (sandy soil L 32, the same moisture content and incubation temperature of 23 C) both fertilizers were completely ammonified after 24 hr of incubation.

The same, however, did not hold for the *nitrification* of the two fertilizers. Here the nitrification in soil (L 32) of the same quantity of nitrogen in the form of ammonium sulphate was compared with Ca-cyanamide extract in which the excess of calcium hydroxide had been removed. The results of this experiment are given in Figs. 5 and 6.

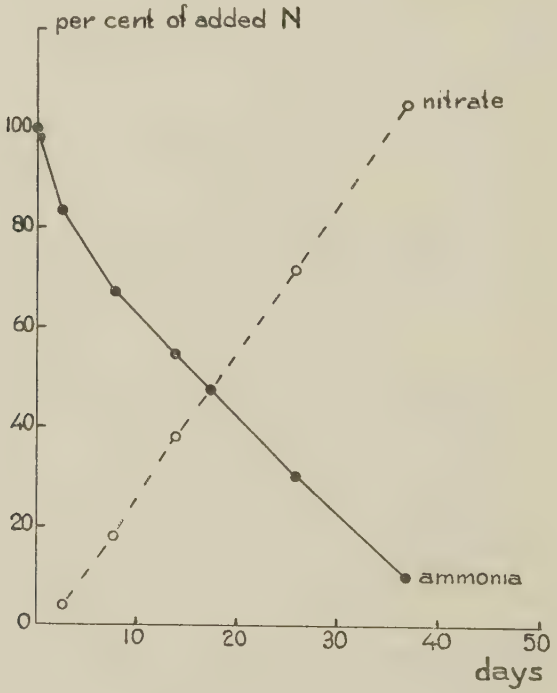

Fig. 5. Nitrification of ammonium sulphate in soil L 32. (90 kg N per ha).

Under the conditions of the experiment, ammonium sulphate was nitrified within 5–6 weeks. In the same time nearly 55% of the added Ca-cyanamide was converted into nitrate. The nitrification of Ca-cyanamide is retarded by the "cyanamide-shock". The possibility remains that this retardation is also influenced by the small quantity of dicyanodiamide, which is probably present in the cyanamide extract. This could not be proved because it was not possible to estimate dicyanodiamide in the extracts and soils.

After approximately 16 days the speed with which the cyanamide is nitrified equals that of the nitrification of ammonium sulphate.

Probably the whole quantity of cyanamide will be nitrified 2–3 week later than that of ammonium sulphate.

The preceding experiments dealt with the nitrification of a cyanamide extract. What will happen when the, in agricultural practice, existing forms of Ca-cyanamide are applied to the soil? Then, gradually a solution of cyanamide will originate and cause a softening of

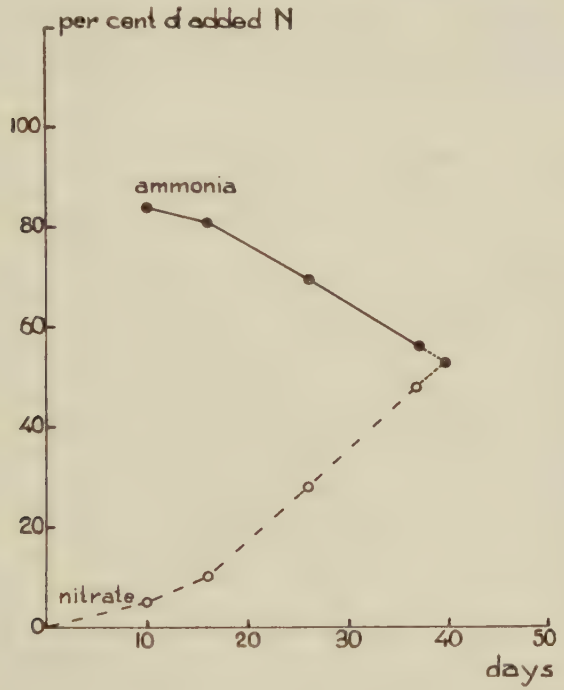

Fig. 6. Ammonification and nitrification of cyanamide extract in soil L 32. (80 kg N per ha).

the "cyanamide-shock". The cyanamide solution comes in contact with the colloidal part of the soil and is transformed into urea. The urea is then hydrolysed by the ammonifying micro-organisms. After this the nitrifying organisms will bring the transformation process to a close.

In the papers dealing with the transformation and decomposition of Ca-cyanamide in the soil several communications can be found concerning very grave retardations of the nitrification of Ca-cyanamide in agricultural soils. In many cases this phenomenon is due to

the fact that most of the investigators added to the soil too much of the fertilizer [25]). Moreover, it is certain that there exist soils which are unfit for fertilizing with Ca-cyanamide. This will be the case with soils low in colloidal materials and micro-organisms. Nitrification of Ca-cyanamide will also be influenced to a great extent by the moisture content, temperature and pH of the soil [12]). As far as the pH is concerned, it is clear that in some cases (very acid soils) Ca-cyanamide will be nitrified better than ammonium sulphate since the lime content of Ca-cyanamide will cause a pH, which is more favourable to the nitrifying micro-organisms.

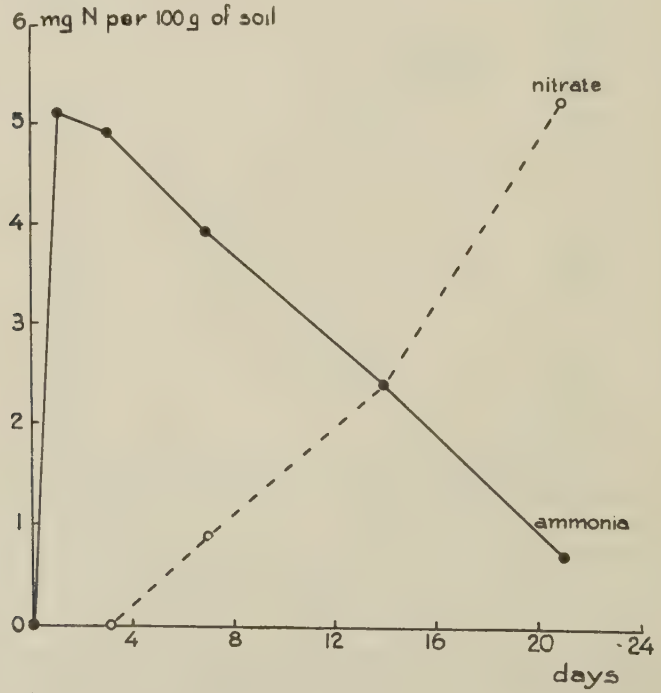

Fig. 7. Ammonification and nitrification of calcium cyanamide in soil.

In judging the use of Ca-cyanamide in agricultural practice as a nitrogen fertilizer, all these facts must be considered. The most important are the concentration of Ca-cyanamide in the soil and the ability of the soil for fertilizing with Ca-cyanamide. These connected properties must be determined for each type of soil which is to be dressed with Ca-cyanamide. Fig. 7 shows that a quantity of 30–40 kg

of nitrogen per ha in the form of Ca-cyanamide will be quickly nitrified in the type of soil corresponding with the sandy soil L 32. After 3-4 weeks incubation at 23°C the Ca-cyanamide was totally nitrified. Earlier a statement of P r a n k e was mentioned about "good agricultural practice" in connection with the danger of the formation of dicyanodiamide when manuring the soil with Ca-cyanamide. The importance of applying the correct amount of fertilizer needed by the particular soil has already been mentioned. The other conditions under which Ca-cyanamide will give its optimal effect are:

1. Fresh Ca-cyanamide of good quality should be used. Storage under humid conditions will promote the formation of dicyanodiamide. Ca-cyanamide has, therefore, to be kept dry and a long storage must be avoided.

2. Ca-cyanamide should be thoroughly mixed with the soil. A contradiction exists when Ca-cyanamide is used as a weed killer. Then, Ca-cyanamide must remain on top of the soil. The risk exists that, in the thin layer of soil, cyanamide is polymerised into dicyanodiamide. The fertilizing effect of Ca-cyanamide will thus be unfavourable. To obtain both the weed-killing action and the fertilizing effect of Ca-cyanamide the fertilizer should be incorporated with the soil after it has remained on top of the soil for 2 or 3 days.

3. The soil to be manured should be of a sufficient moisture content.

4. The temperature of the soil should be at least 10°C.

5. There should exist an active microflora in the soil.

In considering these propositions, it will be seen, from the results obtained, that Ca-cyanamide is a satisfying nitrogen fertilizer, particularly on soils having a low calcium content. As the fertilizer contains 20–21% of nitrogen and 70% hydrated lime it is obvious that this last compound may have a favourable influence on the nitrification of cyanamide in acid soils.

Summary

It is generally assumed that Ca-cyanamide in the soil is converted into ammonia in the following stages:

1. Ca-cyanamide hydrolyse into cyanamide and Ca-hydroxide.
2. Cyanamide is subsequently hydrolysed into urea.
3. Urea is finally converted into ammonium carbonate by micro-organisms.

Two conflicting views exist as to the mode by which urea originates from cyanamide, viz.:

1. Soil induces by means of physico-chemical action the conversion of cyanamide into urea. In this conversion micro-organisms either play no part or a minor one.

2. Urea is formed out of cyanamide by micro-organisms. The physico-chemical hydrolysis of cyanamide is of minor importance when compared with the biochemical hydrolysis.

The data available in the literature did not furnish sufficient proof as to the validity of either assumption, so further investigation was needed.

By means of a method worked out for this purpose it was shown that in the hydrolysis of cyanamide into urea micro-organisms might be completely lacking. Thus the former of the both views cited above is deemed the right one. In the decomposition of cyanamide in the soil the micro-organisms do not play any part before the formation of urea by physico-chemical means has been accomplished.

From literature it may be concluded, however, that many micro-organisms can use cyanamide as a source of nitrogen, provided that a source of carbon is offered as well. The fungus *Aspergillus niger* appears to be able to convert cyanamide rapidly into urea. Although many soil bacteria develop well in a solution which contains cyanamide as a source of nitrogen the rate of the bacterial hydrolysis of cyanamide into urea is small compared with the rate of hydrolysis by physico-chemical means.

When good agricultural soil has been fertilized with Ca-cyanamide, the cyanamide will be converted into urea at such a rate that the micro-organisms will not take any part in the conversion. Particularly because the micro-flora has to adapt itself to the Ca-cyanamide since following the "cyanamide-shock" an initial decrease in the number of micro-organisms in the soil will be induced. This decrease will be soon followed by a large increase. When the micro-flora has adapted itself, however, the cyanamide will have disappeared. The action of the micro-organisms begins with the hydrolysis of the formed urea into ammonium carbonate, while the nitrifying bacteria will bring the mineralisation process to its close. The „cyanamide-shock" which is stronger after the supply of an aqueous extract of Ca-cyanamide than after a manuring with Ca-cyanamide itself, is somewhat injurious for the nitrifying bacteria. The degree of this injury is related to the state of the soil. Depending on the amount of fertilizer supplied the nitrification of Ca-cyanamide will begin some days later than the nitrification of ammonium sulphate or urea. The nitrifying flora will, however, suffer more injury from the dicyanodiamide which may be formed in soil by polymerisation of cyanamid. Although dicyanodiamide is formed in Ca-cyanamide stored under moist conditions, not a single example is offered in literature of formation of dicyanodiamide from cyanamide after the dressing of agricultural soils with Ca-cyanamide. The problem of dicyanodiamide is thus more of theoretical than of practical significance, provided that the Ca-cyanamide remains for as short a time as possible on the top of the soil.

In the Netherlands the slowly acting fertilizer Ca-cyanamide is mainly used as a weed killer. In order to develop both the weed-killing action and the supply with nitrogen it is proposed to leave Ca-cyanamide for as short a time as possible on the top of the soil. It is expected that all soils in good agricultural condition will prove good Ca-cyanamide soils. The optimal concentration of the fertilizer will vary, however, for the various types of soil.

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ESSAI D'UNE THÉORIE DE LA STABILITÉ DE LA STRUCTURE DES SOLS

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Le problème de la stabilité de la structure a fait l'objet de nombreuses recherches; des théories souvent contradictoires ont été proposées pour interpréter de petits groupes de résultats ou même des résultats isolés. Devant cet état de faits, nous avons pensé qu'il serait utile de jeter les bases d'une théorie ayant l'ambition de faire la synthèse des divers résultats obtenus. En 1938, nous avons, dans un premier mémoire, réalisé cette tentative [5]; nous nous proposons actuellement de rappeler les résultats obtenus dans ce premier travail et de montrer comment les recherches que nous avons entreprises depuis cette époque viennent confirmer nos conclusions antérieures.

Nous rappellerons que 2 hypothèses ont été formulées pour expliquer la façon dont la structure se dégrade:

La première due à Schloesing [10] suppose qu'au contact de l'eau, les ciments agglomérant le squelette sableux, dont l'ensemble constitue le sol, peuvent se disperser dans certaines conditions qui correspondent évidemment au conditions de dispersion des colloïdes. Cette théorie cadre parfaitement avec ce que l'on sait de l'action des sels de sodium et, dans une certaine mesure, de l'action de la chaux sur les propriétés physiques des sols. Par contre, l'influence de la matière organique s'interprète moins aisément. D'autre part, si, comme différents auteurs ont essayé de le faire, on veut juger de la stabilité de la structure en déterminant la quantité d'éléments fins qui se dispersent à partir d'un échantillon de terre agité dans l'eau distillée, on constate pour différents types de sols que le résultat obtenu n'est pas du tout en accord avec le comportement de ces mêmes terres au champ. On peut donc conclure que si la théorie de Schloesing est valable, ce n'est que pour un certain nombre de cas particuliers; il faut donc chercher une 2e interprétation.

Différents auteurs dont Yoder [12] ont admis que la structure se dégradait de la manière suivante: quand un agglomérat de terre est immergé dans l'eau, le liquide tend à pénétrer dans les pores du sol

sous l'influence des forces capillaires. L'atmosphère qui est à l'intérieur se trouve comprimée et, lorsque la pression de l'air est supérieure à la cohésion des particules de terre, celles-ci éclatent et le sol se délite.

Il est évidemment difficile de juger de la stabilité d'un sol. Il y a très peu de terres qui, une fois plongées dans l'eau, ne se délitent pas plus ou moins, à condition de partir d'éléments assez gros. Les méthodes utilisées pour juger de la stabilité de la structure doivent partir d'éléments comparables et c'est pourquoi nous avons adopté une méthode dans laquelle on utilise un échantillon préparé à peu près comme pour une analyse mécanique. Dans ces conditions, certaines terres paraissent avoir une stabilité totale. Cette technique n'est pas absolument arbitraire car si l'on considère des volumes de terre de plus en plus grands, prélevés dans le sol en place, on constate que la cohésion varie d'une façon tout à fait irrégulière car elle dépend de la présence de failles ou de craquelures et la probabilité qu'un tel accident se rencontre croît évidemment, toutes choses égales d'ailleurs, avec le volume de terre considéré.

Nous allons donc établir le raisonnement théorique qui suit, en le rapportant non pas au comportement exact du sol en place, mais à un schéma simplifié du même sol constitué par l'échantillon préparé comme nous l'avons indiqué précédemment:

Posons:

C, la cohésion du sol mesurée empiriquement par la résistance à la rupture de fragments de terre.
α, l'angle de raccordement sol/eau.
r, le diamètre des plus gros capillaires du sol.
T_1, la tension superficielle du sol.
T_2, „ „ „ de l'eau.
T_{12}, la tension interfaciale sol/eau.

A l'affinité sol-eau.

Notre hypothèse [5]) peut s'exprimer de la façon suivante, si S mesure la stabilité:

$$S = C - \frac{2T_2 \cos \alpha}{r} \qquad \text{(I)}$$

mais

$$\cos \alpha = \frac{T_1 - T_{12}}{T_2} \qquad \text{(II)}$$

d'où

$$S = \frac{rC - 2(T_1 - T_{12})}{r} \qquad \text{(III)}$$

enfin si $$T_{12} = T_1 + T_2 - A \qquad \text{(IV)}$$

il vient $$S = \frac{rC + 2T_2 - 2A}{r} \qquad \text{(V)}$$

et la condition d'éclatement s'écrit: $rC + 2T_2 < 2A$.

Si nous considérons la formule IV nous pouvons écrire $2T_1 + 2T_2 < 2A$, dans ce cas T_{12} est négatif: on interprète ce fait en disant que le colloïde tend à se disperser; on voit donc (form. III) que, rC étant plus petit que $2T_1$ la terre éclate avant de se disperser, les deux phénomènes ne jouent pas dans la même gamme de valeurs.

Mais l'introduction de la formule (II) dans la formule (I) n'a de sens que si $-1 < \cos a < +1$.

Remplaçons T_{12} dans (II) par sa valeur tirée de (IV) il vient

$$\cos a = \frac{T_1 - T_1 - T_2 + A}{T_2} = \frac{A - T_2}{T_2}$$

soit au maximum, si $\cos a = 1$, $T_2 = A - T_2$ ou $2T_2 = A$. En conséquence, même si A est très grand il ne peut y avoir éclatement si $rc < 2T_2$.

On peut alors dresser le tableau suivant:

conditions physico-chim.	Phénomènes observés			cas pratique correspondant
	stabilité	éclatement	dispersion	
a) $rC + 2T_2 > 2A$	+	0	0	terre non mouillable
b) $rC + 2T_2 < 2A < 2T_1 + 2T_2$				
1°) $rC > 2T_2$	+	0	0	terre très cohérente mais mouillable
2°) $rC < 2T_2$	0	+	0	terre mouillable, peu cohérente
c) $2A > rC + 2T_2 > 2T_1 + 2T_2$				
1°) $rC < 2T_2$	0	+	+	terre peu cohérente à ciment dispersable
2°) $rC > 2T_2$	0	0	+	terre cohérente à ciment dispersable

Le simple résultat qualitatif que représente le tableau précédent montre que les déductions tirées des calculs doivent être en gros applicables. Cependant, il ne faut pas se dissimuler le caractère hypothétique de cette tentative car beaucoup de formules utilisées ont été extrapolées du cas des liquides pour lesquels elles ont été vérifiées au cas des solides, où leur vérification est extrêmement difficile. Nous allons donc considérer ces déductions comme des hypothèses et cher-

cher à les vérifier expérimentalement, en reprenant un par un chacun des facteurs qui ont été mis en cause.

Influence de la cohésion. Les calculs établis précédemment montrent que la stabilité d'une terre croît avec sa cohésion. Ces résultats ont été vérifiés en particulier par les travaux de Vilensky [11]) et par nous-même [5]). Les expériences montrent que si l'on accroît la cohésion d'une terre par pétrissage dans des conditions convenables, la stabilité de la structure, cependant mesurée par des méthodes différentes — la technique de Vilensky étant d'un autre type que la nôtre — est accrue.

D'autre part, nous avons pu monter [5]) qu'en floculant des doses croissantes d'une même argile sur une même quantité d'un même sable extrait d'un sol, on accroissait à la fois la cohésion et la stabilité du système. Une remarque est à faire cependant: pour les faibles teneurs en argile, le délitement conduit à des particules constituées soit par le sable lui-même, soit par de petits agglomérats d'argile ayant à peu près la même dimension que les grains de sable; si l'on accroît la teneur en argile de façon que les grains de sable soient noyés dans l'argile, on obtient des agglomérats relativement gros qui restent stables, ils sont constitués par une pâte d'argile enrobant le squelette sableux. Récemment, Peterson [9]) a obtenu des résultats analogues avec 2 types d'argile différents, la kaolinite et la montmorillonite. Cet auteur a constaté qu'à quantités d'argile égales, la montmorillonite formait des aggrégats beaucoup plus stables que la kaolinite mais le sens général du résultat reste le même.

Une autre vérification de la théorie est donnée par le résultat suivant: une terre sodique assez argileuse donne à l'analyse des aggrégats d'une part, des éléments grossiers qui restent stables vis-à-vis du phénomène d'éclatement — et des éléments très fins provenant de la dispersion de l'argile.

Notons également que des terres constituées par un sable fin et contenant peu d'argile ne se dispersent pas si elles sont calciques. Cependant, leur délitement donne un milieu suffisamment compact pour qu'il constitue un sol de mauvaise qualité culturale. C'est, en particulier, le cas des terres "battantes". Une dernière remarque: puisque le phénomène d'éclatement est limité aux sols de faible teneur en argile, il est inutile de poursuivre l'étude expérimentale de ce phénomène, en particulier d'étudier l'influence de la mouillabilité ou de la teneur en air sur des terres trop cohérentes. C'est probable-

ment pour avoir travaillé avec un matériel mal choisi que divers auteurs trouvent des résultats contradictoires.

Influence de la teneur en air. Il s'agit de mettre en évidence le rôle joué par l'atmosphère contenue dans les aggrégats. On peut opérer de 3 manières différentes.

Il est possible de partir d'un échantillon de terre humide de le tamiser, puis de le laisser sécher lentement, et à divers états d'humidité, d'en prélever une fraction pour fair une analyse d'aggrégats. On constate alors qu'à mesure que le sol se dessèche, la proportion d'éléments grossiers obtenus à l'analyse décroît. Ce résultat a été obtenu par Achromeiko[1]) et a pu être établi à nouveau dans notre laboratoire [5]).

On peut, inversement, imbiber la terre avec précaution, de manière à ce que l'air soit chassé au lieu d'être emprisonné par l'eau comme cela se produit quand l'humectation est trop rapide. Dans ces conditions, on observe toujours une augmentation de la proportion d'éléments grossiers quand on opère sur de la terre qui a été mouillée avec précaution et que l'on compare avec les résultats fournis par l'humectation directe.

Notons toutefois qu'il faut éviter, quand les terres sont de faible cohésion, de faire subir des actions mécaniques à la terre mouillée car on peut alors détruire les aggrégats ce qui diminue beaucoup les différences observées.

Notons également que ce fait nous indique qu'il faut soigneusement définir les conditions dans lesquelles on humecte la terre chaque fois qu'on fait une analyse d'aggrégats; autrement, les résultats ne sont pas reproductibles.

Mais la manière la plus directe de vérifier l'influence de la teneur en air est encore d'opérer en mouillant la terre sous un vide plus ou moins poussé ou en opérant sous pression.

Voici les résultats qui ont été obtenus dans notre laboratoire [6]):

p. cent d'aggrégats > 0,2 mm obtenus par mouillage dans une atmosphère à la pression de:				
	0.01 at.	0.25 at.	1 at.	3 at.
Sablière — Horizon *B*	32	19	10	6
Limon rouge	76	51.5	18	4
Versailles — sol cultivé	62.5	44.0	20	7
,, — forêt (Hor. *A*)	—	85.0	63.5	40
Sol Stat. Clermont-Ferrand	–	—	94.0	90

On constate que la destruction des agglomérats est d'autant plus intense qu'ils sont mouillés dans une atmosphère plus comprimée ce qui est conforme à l'hypothèse.

Cependant, il est possible d'objecter que les agglomérats mouillés sous pression sont détruits au moment où l'on ouvre le récipient contenant la terre, l'atmosphère à l'intérieur des agglomérats se détendant brusquement. On peut éviter cette critique en remplaçant l'air par un liquide non miscible à l'eau tel que CCl_4 ou CS_2, etc. Dans ces conditions, l'atmosphère de l'agglomérat est pratiquement incompressible. Voici les résultats obtenus:

	p. cent d'aggrégats > 0.2 mm					
					Versailles	
	Latérite (Argentine)	Sol à croûte (Algérie)	(*B*) cultivé		(*A*) très humique	(*A*) très humique
	1	2	3	4	5	6
sans prétraitement	54	33.5	20	17	67	69.5
avec CS_2	—	—	4	2	61	—
avec CCl_4	2	8	5.5	3.5	65.5	79
avec C_6H_6	2	6	—	3	67	78
avec huile de vaseline	—	—	4.5	—	63	—

On constate que tous les sols de 1 à 4 sont effectivement sensibilisés à l'action de l'eau comme il était prévu. Au contraire, 5 et 6 ne voient pas leur stabilité diminuée et la terre 6 est même légèrement plus stable. Nous discuterons plus loin ce résultat.

Auparavant, nous citerons une autre expérience réalisée pour montrer que le phénomène est le même en présence de liquide non miscible à l'eau qu'en présence d'air. Dans ce but, le liquide a été déplacé en mouillant par capillarité avec de l'eau la terre déjà imbibée de liquide organique. Les résultats obtenus sont reproduits dans le table au ci-après:

	p. cent d'aggrégats > 0.2 mm			
	Terre sèche	Terre imbibée d'eau	Terre imbibée de CCl_4	Terre imbibée de CCl_4 puis mouillée par capillarité
	1	2	3	4
Longwy (s-sol)	50	55	15	26
Limon rouge	12.5	35	6	14.5
,, argileux	34	50	6	20

On constate que le déplacement du liquide organique par imbibition

capillaire accroît la stabilité par rapport à l'imbibition par CCl_4. Ceci confirme notre hypothèse. Cependant les résultats restent inférieurs à ceux obtenus avec la terre sèche. Le fait que l'on n'obtienne pas par imbibition capillaire (colonne 4) une stabilité analogue à celle obtenue pour la terre sèche (colonne 1) s'explique aisément, car il est facile de voir que, malgré les précautions employées, une partie des aggrégats est détruite lors de l'imbibition capillaire, l'eau emprisonnant toujours un peu du liquide organique. On peut également objecter que la tension superficielle au contact entre l'eau et le liquide organique n'est pas la même que celle de l'eau pure. Mais nous avons vu dans nos calculs que la tension superficielle de l'eau n'intervenait pas dans le phénomène; nous avons d'ailleurs vérifié expérimentalement qu'en effectuant une analyse d'aggrégats avec de l'eau saturée par un corps tensio-actif, on obtenait sensiblement le même résultat qu'avec l'eau pure, du moins si le corps était peu soluble. Si le corps est très soluble, tel l'alcool éthylique, on constate que pour les concentrations élevées, la stabilité de la terre croît notablement. En analysant le phénomène, par comparaison des courbes représentant en fonction de la composition du mélange, les variations de la tension superficielle, de l'énergie libre de l'eau, et de la stabilité de la terre, il a été possible d'établir que c'était la diminution de l'énergie libre de l'eau qui était cause de l'accroissement de la stabilité du sol. D'ailleurs, on peut obtenir un résultat du même ordre, en utilisant des solutions salines dans lesquelles la tension superficielle — par conséquent T_2 — reste sensiblement constante bien que l'énergie libre de l'eau diminue sensiblement. C'est ce qui ressort du tableau suivant:

Nature du soluté	Température d'ébullition (°centigr.) de la solution utilisée pour l'analyse d'aggrégats	p. cent d'aggrégates stables > 0.2 mm — Nature du sol (Versailles)		
		Hor. *A*	Hor. *B*	Hor. *C*
NO_3NH_4	112	40	26	10
	105	29	20	7.5
	102	26.5	18	6
	100	24.5	10	4
Cl_2Mg	118	43	32	10
	103	26.5	12	6
	100	24	9	4
Cl_4Ca	111	32	20	9
	102	25	15	6
	100	24	9	4

On constate donc que la stabilité croît avec la température d'ébullition du liquide, c'est-à-dire quand l'énergie libre de l'eau décroît, ainsi que le veulent nos hypothèses. Cependant, il semble bien qu'il y ait un effet différent d'une solution à l'autre. Ceci prouverait qu'il y a une action spécifique du soluté, ce qui n'est nullement en opposition avec les hypothèses.

Par ailleurs, le point de départ des interprétations théoriques repose sur l'existence de deux phénomènes: l'un dû à des phénomènes capillaires, l'autre à un phénomène de dispersion. Or, l'expérience courante nous montre que les argiles floculent pour des concentrations en électrolytes beaucoup plus faibles que celles qui sont nécessaires pour assurer la stabilité des agrégats grossiers. D'autre part, l'action spécifique des ions notée précédemment est tout à fait différente de celle qui se manifeste lors de la floculation. Ceci prouve bien que le phénomène d'éclatement est différent du phénomène de dispersion.

Si nous revenons maintenant au fait que l'imbibition par CCl_4 ne sensibilise pas les terres riches en matière organique au traitement ultérieur par l'eau, nous pouvons rapprocher cette constatation de l'accroissement de stabilité observé lors de l'imbibition préalable de l'échantillon par capillarité. Il existe une différence cependant entre les 2 essais: c'est que l'eau est miscible à elle-même tandis que le tétrachlorure de Carbone est insoluble. On peut alors supposer que le corps qui joue un rôle protecteur doit son action au fait qu'il est fixé par une partie au moins des constituants du sol. Dans le cas du prétraitement par les liquides organiques, ce serait la fixation du liquide organique par la matière humique du sol qui le protégerait contre l'action ultérieure de l'eau.

Pour vérifier cette hypothèse, nous avons imbibé une série de terres [7]) par divers liquides organiques miscibles ou non à l'eau et possédant des fonctions chimiques différentes. Les résultats sont consignés dans le tableau suivant: (page 175).

Nous constatons ainsi que l'eau, les fonctions alcool, acétone, éther oxyde sont protectrices dans toutes les terres non sodiques; à ces fonctions nous pouvons ajouter la fonction amine, acide, aldéhyde et les éthers-sels. La masse de la molécule semble abaisser l'activité de la fonction.

Rappelons que l'efficacité de ces fonctions correspond au classement général de l'activité de ces liquides vis-à-vis des diverses propriétés physiques des sols: chaleur d'humectation, densité apparente,

Liquide de prétraitement	p. cent d'aggrégats > 0.2 mm				
	Versailles B	Sol argileux	Versailles A Humique	Terre 1 rendue sodique	Terre 3 rendue sodique
	1	2	3	4	5
Eau.	33.5	49.5	37.5	2.5	2
Alcool méthylique . .	31.5	53.5	35.5	5.5	2
Alcool isobutylique . .	34	46	38.5	—	1.5
Alcool laurique. . . .	10	14.5	—	—	2
Acétone	33.1	48	35	—	
Ether	26	32	26	-	—
Nitrobenzène.	9.5	7	32	2	14.5
Tétrachlorure	7.5	6	21	3	14

gonflement [5]). Nous sommes donc en droit de dire qu'il s'agit bien de réaction avec le sol et d'une diminution de l'affinité.

Pour appuyer cette affirmation sur une autre donnée, nous avons mesuré [5]) la cohésion de bâtonnets de terre sèche après imbibition dans ces mêmes liquides, et nous avons constaté que les liquides qui abaissent le plus la cohésion sont les plus protecteurs quand ils sont fixés avant l'imbition par l'eau.

Enfin nous avons constaté un fendillement des bâtonnets très intense pour les liquides les plus protecteurs alors que ce phénomène n'existe pas avec les liquides non protecteurs. On voit à nouveau la similitude de comportement des liquides classés comme protecteurs et de l'eau.

Ceci permet encore de conclure que l'action protectrice n'appartient qu'aux liquides ayant une affinité pour la terre et l'on conçoit alors que le système liquide sol ainsi constitué ait moins d'affinité pour l'eau que le sol sec.

On constate également qu'il n'est pas indispensable que le liquide de pré-traitement soit miscible à l'eau. Il n'est pas nécessaire non plus que tous les pores du sol soient remplis du liquide. Nous avons montré antérieurement [3]) que la fixation d'une petite quantité de matière grasse suffit à assurer un accroissement de stabilité considérable: 2% d'une huile végétale suffisent pour assurer ce résultat. Dans ces conditions, on constate d'ailleurs que la terre flotte sur l'eau: ceci montre sa faible mouillabilité et ce résultat s'interprète directement dans les formules comme une diminution de l'angle de raccordement sol eau ou, en d'autres termes, comme un abaissement de la pression capillaire. Dans la nature, ce sont les matières organiques du sol qui jouent le rôle de ces matières grasses.

Ainsi, l'ensemble des expériences entreprises vérifie les déductions théoriques cependant fragiles établies au début de ce mémoire. Il en résulte qu'un des mécanismes de destruction de la structure est bien conforme à la 2e hypothèse. Cependant, le point de vue de Schloesing [10]) est aussi confirmé et l'on sait bien qu'une terre dont le complexe est saturé par des cations monovolents, du sodium en particulier, voit sa stabilité diminuée. On a parfois avancé, et nous avons nous-mêmes admis, que le gonflement des colloïdes accompagnant leur hydratation était aussi une cause de destruction des agglomérats terreux; sans nier la possibilité d'intervention de ce phénomène, nous considérons dans l'état actuel des expériences qu'il n'est pas nécessaire d'en tenir compte.

On constate également que les 2 mécanismes supposés: éclatement et dispersion jouent dans des conditions très différentes. Ainsi, une terre à éléments fins pauvre en argile a une structure très instable mais ne manifeste pas de dispersion. Au contraire, une terre argileuse sodique donne à l'analyse des aggrégats grossiers mais aussi des éléments fins dispersés. Enfin, les concentrations en sels nécessaires pour assurer la stabilité des aggrégats grossiers sont très supérieures à celles qui sont nécessaires pour provoquer la floculation des colloïdes.

Cependant, les 2 hypothèses proposées paraissent encore insuffisantes; en effet, nous avons pu constater dans la nature que les apports d'amendements calcaires améliorent très nettement les propriétés structurales telles que la perméabilité, la porosité, la cohésion, etc. [3]). Or, l'élévation du pH de terres artificielles par addition d'eau de chaux, l'addition de carbonate de calcium, lors de l'analyse des aggrégats, ne modifient pas la stabilité de la structure. D'autre part, les analyses d'aggrégats sur des terres améliorées par des apports d'amendements calcaires ne font pas toujours apparaître cet accroissement de stabilité. Il est évident d'ailleurs, étant donné la faible solubilité de ces substances que leur présence ne peut pas modifier le phénomène d'éclatement. On a imaginé que ces amendements, en modifiant la distribution des matières humiques, pouvaient ainsi les rendre plus efficaces [4]). Cette conception nous paraît erronée pour la raison suivante: si l'on compare le comportement d'une terre enrichie en matière organique après pré-traitement par divers liquides et son témoin, on constate un accroissement net de stabilité. Si l'on effectue le même essai avec une terre chaulée et la terre témoin, on constate une diminution de stabilité; c'est ce que montre le tableau suivant:

Terres utilisées	Matières organiques différence ‰ entre 1 et 2	Traitements (Les résultats sont exprimés en % de particules supérieures à 0.2 mm par rapport au traitement à l'alcool)					Somme algébrique des différences par chaque traitement
		tétrachlorure de carbone	nitrobenzène	chlorure de méthyle	acétate d'amyle	alcool éthylique	
Parcelles fumées Versailles							
A1 Fumier		40	60	85	65	100	
A2 Témoin	13.4	11	42	46	42	100	
Différence A1—A2		+29	+18	+39	+23		+109
En luzerne 6 ans							
B1 En luzerne		29	52	62	80	100	
B2 Témoin	1.8	17	29	57	50	,,	
Différence B1—B2		+12	+23	+ 5	+30		+70
Terre chaulée							
C1 Carbonate de chaux	0	20	34	57	54	100	
C2 Témoin		28	55	55	50	,,	
Différence C1—C2		— 8	—21	+ 2	+ 4		—23

Ces quelques exemples pris parmi une douzaine tous dans le même sens et portant sur des terres dont le comportement au champ était connu semblent bien établir que le mécanisme de l'amélioration de la structure par apport d'amendements calcaires est différent de l'effet de la fumure organique.

On peut réaliser une expérience exactement inverse dans laquelle on fait apparaître un accroissement de stabilité pour les terres chaulées par rapport aux terres fumées. Il suffit pour cela de laisser sédimenter des suspensions de terre très concentrées par exemple, contenant 400 gr par litre de substances solides. On voit alors la suspension de terre chaulée s'éclaircir très rapidement alors que la suspension de terre fumée ne se décante que lentement. Ces effects ne s'observent pas ou sont beaucoup moins nets si l'on opère sur des terres très diluées dans l'eau. Il semble donc y avoir un 3e mécanisme vis-à-vis duquel l'effet du chaulage se manifeste. L'influence de ces substances apparait également quand on fait des mesures de percolation au travers des échantillons de terre. Il suffit même d'ajouter 1% de carbonate de calcium du commerce intimement mélangé à l'échantillon au moment de l'essai pour voir la vitesse de filtration accrue d'une façon significative. S c h l o e s i n g dans des expériences très

démonstratives qu'il avait effectuées pour établir l'action de la chaux, opérait également en milieu concentré. Il semble donc que, dans ce phénomène, on met en évidence une action des particules à petite distance.

Quoi qu'il en soit, nous croyons à la suite de ces études, qu'il est nécessaire pour déterminer la stabilité d'une structure, d'utiliser non pas une méthode, mais une série de méthodes, chacune d'entre elles mettant en évidence l'influence d'un des 3 facteurs considérés. Bien que la mise au point de ces méthodes ne soit pas encore réalisée, il a été possible, en utilisant les procédés indiqués, de guider utilement les praticiens. Et d'ailleurs, si ces considérations sont exactes, c'est encore leur application au milieu naturel et la réussite des prévisions qui permettra de les vérifier. Nous pensons en effet que la mise au point de techniques de contrôle est l'un des aboutissements de tout travail théorique qui trouve ainsi sa vérification et sa justification.

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INVESTIGATIONS ON THE NITROGEN NUTRITION OF PEA PLANTS

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Introduction. Pea plants, like other members of the *Leguminosae*, generally derive the nitrogen required for their proteins and other nitrogenous compounds from the gaseous nitrogen fixed by the nodules. Extensive investigations on the biochemistry of the nitrogen fixation have been carried out by Virtanen *et al.* [8], [9] and by Wilson *et al.* [10].

The question whether the nodules of leguminous plants are fully capable of supplying their hosts with nitrogen fixed from the atmosphere, or that an additional absorption of combined nitrogen from the soil may still improve the growth of the plants, is important not only from a theorethical point of view but also in respect of the growing of these crops in practice.

The bad results sometimes obtained by Dutch farmers with the growing of peas, gave rise to an investigation of the effect of nitrogenous fertilizers on the growth of pea plants on certain soils. In addition to field experiments, investigations with pea and oat plants in nutrient solutions with increasing amounts of nitrogen were carried out. In other experiments the value of different nitrogen compounds as a nitrogen source for peas growing in nutrient solutions without *Rhizobium* was determined.

In experiments with nutrient solutions and with soil the influence of zinc and boron on the fixation of gaseous nitrogen was investigated.

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Experimental methods. In the experiments with culture solutions a medium of the following composition was used:

distilled water . . .	1 l	$MnSO_4.4H_2O$ . . .	1 mg
KH_2PO_4	325 mg	$ZnSO_4.7H_2O$ . . .	0.25 mg
Na_2HPO_4	70 mg	$CuSO_4.5H_2O$ . . .	0.25 mg
$MgSO_4.7H_2O$	250 mg	H_3BO_3	0.25 mg
$CaSO_4.2H_2O$	250 mg	$Na_2MoO_4.2H_2O$ . .	0.05 mg
$FeCl_3.6H_2O$	25 mg		

In preparing these solutions water distilled in a glass apparatus was employed in most cases.

The pea seeds were germinated in quarz sand previously cleaned with nitric acid and washed free from acid with distilled water. The nutrient solutions were periodically aerated. They were changed every three or four weeks.

Effect of different amounts of anorganic nitrogen compounds on the growth of pea plants in nutrient solutions. In order to elucidate the behaviour of pea plants to anorganic nitrogen compounds, experiments with culture solutions supplied with different amounts of ammonium nitrate were carried out. „Mansholt" peas were transplanted into glass jars of 700 cc capacity on April 18, 1942, each jar holding three plants. On April 25 ammonium nitrate was added in amounts of 0, 20, 50, 100 and 200 mg N per jar respectively.

In order to prevent the development of nodules the jars and the nutrient solutions as well as the pea seeds were disinfected. Although no special precautions were taken in this experiment to keep the solutions free from spontaneous infection, no nodules developed.

On May 1 the first symptoms of nitrogen deficiency were observed: a light green shade of the leaves and a decrease in growth. On May 11 a good many of the lower leaves of the nitrogen-deficient plants had died, the dead tissue showing a gray white colour.

At the latter date one set of cultures was harvested. The fresh weights were determined and the plants were analysed for some nitrogenous compounds. Of the remaining cultures the nutrient solution was changed; the concentration of the nutrient salts was doubled, except that of ammonium nitrate which was given in the same amounts as before.

Some plants grown without nitrogen and some with 20 mg of

nitrogen were supplied with 23.3 mg of nitrogen in the form of ammonium sulphate and as calcium nitrate respectively, to find out to what extent nitrogen-deficient pea plants respond to anorganic nitrogen compounds. Precautions were taken to prevent an acidification and an alkalinisation of the culture solution by these salts.

On May 21 a second set of pea plants was harvested and on May 28 a third. The fresh weights of the plants are recorded in table I. At both dates again some nitrogen-deficient plants were supplied with 23.3 mg nitrogen in the form of ammonium sulphate and calcium nitrate respectively.

TABLE I

Influence of nitrogen on the yield of pea and oat plants

mg of nitrogen per jar *)	Time of harvest	Peas				Oats			
		Fresh weights in g		Dry weights in g		Fresh weights in g		Dry weights in g	
		Leaves + stems	Roots	Leaves + stems	Roots	Leaves + stems	Roots	Leaves + stems	Roots
0	May 21(20)	1.66	6.02	—	—	0.34†)		—	—
20	,,	5.79	8.59	—	—	4.03	2.94	—	—
50	,,	9.15	9.96	—	—	8.26	3.92	—	—
100	,,	14.06	8.76	—	—	11.21	3.91	—	—
200	,,	15.—	10.58	—	—	10.62	3.89	—	—
0	May 28	1.25	5.26	0.54	0.33	0.73	0.86	0.14	0.05
20	,,	6.88	9.73	1.25	0.65	9.13	5.76	1.59	0.56
50	,,	10.70	10.75	2.—	0.91	16.33	5.46	2.67	0.50
100	,,	20.36	10.78	3.45	0.93	24.70	9.77	3.20	0.77
200	,,	24.43	10.91	4.88	0.79	21.35	8.61	3.93	0.75

*) these amounts were added two times

†) weight of total plants

On June 10, after photographs of the plants had been taken, the experiment was brought to an end. The pea plants without nitrogen had completely died. The same was true of the greater part of the plants with the low rate of nitrogen (see fig. 1).

These results were in contrast with those of a similar experiment with oat plants which was carried out simultaneously. Although without the addition of nitrogen the development of the oat plants was much poorer than that of the peas, probably due to the much lower amount of nitrogen in the oat seeds, these plants were able

to complete their normal life cycle. Pea plants apparently require a considerably higher nitrogen level in their tissues than oats and other cereals do. When the nitrogen content comes below this level the pea tissue turns yellow and dies shortly afterwards. Nitrogen-deficient oat leaves, however, although showing a yellow colour and often dead tips, remain alive until the seed has ripened.

The different behaviour of pea and oat plants to nitrogen was not only apparent in the deficiency symptoms but also in the response of the deficient plants to newly introduced ammonium and nitrate nitrogen. Pea plants generally responded very badly to the

Fig. 1. Pea plants in nutrient solution without *Rhizobium* with different amounts of ammonium nitrate (1 : without nitrogen, 2 : 40, 3 : 100, 4 : 200 and 5 : 400 mg N per culture; added in two parts).

added nitrogen (see fig. 2 and 3). Leaves showing a light green shade were unable to retake a normal green colour following the addition of the nitrogen compounds. Only young emerging leaves responded to the added nitrogen. The light green leaves of the oat plants, on the contrary, readily turned normal green when the nutrient medium was supplied with nitrogen (see fig. 4).

Analysis of the nitrogen fractions of pea and oat plants grown with different amounts of ammonium nitrate. In order to obtain some information concerning the cause of the above-mentioned differences be-

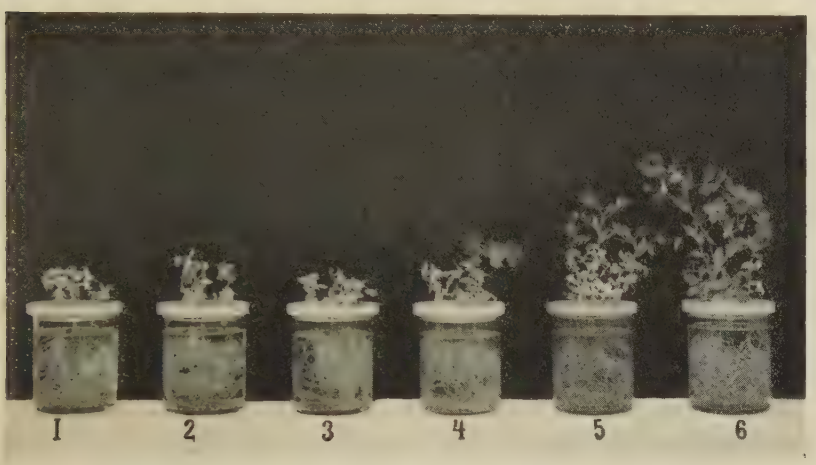

Fig. 2. Nitrogen-deficient pea plants without *Rhizobium*, supplied with 23.3 mg N on May 28 (1 and 2), on May 20 (3 and 4) and on May 13 (5 and 6) To the pots 1, 3 and 5 the nitrogen was added in the form of $(NH_4)_2SO_4$, to 2, 4 and 6 as $Ca(NO_3)_2$. Photograph taken on June 10.

Fig. 3. Pea plants grown with 20 mg of N in the form of NH_4NO_3 without *Rhizobium*; supplied with 23.3 mg N as $(NH_4)_2SO_4$ (5) and $Ca(NO_3)_2$ (6) on May 13. Photograph taken on June 11.

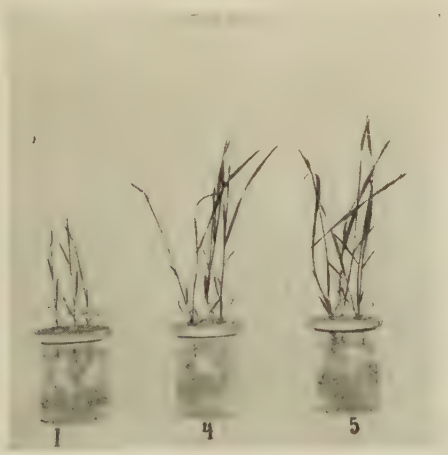

Fig. 4. Nitrogen-deficient oat plants supplied with 23.3 mg N as $(NH_4)_2SO_4$ (pot 4) and $Ca(NO_3)_2$ (pot 5) on May 20. To pot 1 no nitrogen was added. Photograph taken on June 11.

tween pea and oat plants, chemical analyses of the fresh tissues of these plants were carried out.

Proteins were precipitated by heating the ground plant tissue for 15 min. above 90°C and after cooling, adding trichloroacetic acid. Protein and soluble N were separated by filtration and both fractions were analysed for total N according to Kjeldahl–Lauro. Soluble N estimated by this method does not include nitrate-N.

Nitrate-N was determined according to the xylenol method (1).

Ammonia-N was distilled under reduced pressure at 40°C after adding a borate buffer of pH 9.

Amide-N was determined by hydrolysing an aliquot part of the filtrate in 5% H_2SO_4 and distilling the ammonia formed. NH_4-N originally present has to be subtracted.

The results of these analyses are reported in table II.

TABLE II

Nitrogen fractions in mg per 10 g of fresh plant tissue											
mg of nitrogen per jar	Time of harvest	Protein-N		Soluble-N†)		Nitrate-N		Ammonium-N		Amide-N	
		Leaves + stems	Roots	Leaves + stems	Roots	Leaves + stems	Roots	Leaves + stems	Roots	Leaves + stems	Roots
0	Peas	21.1	12.1	10.2	5.9	0	0	1.0	0.4	2.1	0.6
20	May 11	25.5	17.6	6.3	9.2	0	0	0.5	0.3	1,0	1.5
50		32.8	20.5	3.–	12.3	0	0	0.3	0.9	1.5	1.0
100		47.1	18.3	14.8	15.7	0	0.2	0.2	0.9	2.5	1.8
200		48.6	20.9	23.7	16.8	0.3	0.7	0.4	0.9	5.–	2.7
0	Peas	36.3	9.8	19.8	5.–	0	0	0.3	0.1	2.–	0.5
20	May21*)	43.7	14.2	7.8	6.2	0	0.6	0.6	0.4	2.2	0.3
50		52.5	20.9	12.4	11.1	0	0.3	0.3	0.6	—	0.7
100		37.5	24.5	18.1	17.–	0	0.6	0.2	0.6	2.–	0.6
200		58.4	24.7	26.7	21.4	0.1	0.9	0.3	1.1	3.9	1.7
0	Oats	—	—	—	—	—	—	—	—	—	—
20	May20*)	28.3	11.1	8.1	4.3	0	0	0.7	0.7	0.6	1.0
50		35.7	12.2	11.9	5.8	0.3	0	0.3	0.3	0.7	1.9
100		45.3	15.5	17.9	14.8	2.8	0.8	0.3	0.8	3.6	3.7
200		49.3	17.–	24.3	20.1	5.1	1.8	0.6	1.–	5.6	7.3

*) two applications of nitrogen

†) except nitrate

It can be seen that pea plants showing heavy symptoms of nitrogen deficiency are very low in protein nitrogen. The content of soluble organic nitrogen of leaves plus stems, however, is much higher than in plants with a moderate nitrogen supply. Apparently a

considerable part of the proteins is converted into soluble organic nitrogen compounds. Although the breaking down of the proteins in the pea leaves undoubtedly is connected with the dying down of the leaves and stems, it may be a result and not the cause of this process.

Nitrate nitrogen was very low in the pea plants, even when these were growing in solutions with high applications of ammonium nitrate. Apparently after absorption it is readily converted into organic nitrogenous compounds. In oats, however, a considerable accumulation of nitrate in leaves and stems occurred under the same circumstances.

Ammonia-N was very low in all cases.

Amide nitrogen was low in plants supplied with a moderate amount of ammonium nitrate. It increased considerably with higher applications of nitrogen. In oats this rise was more evident than in peas. This was true for leaves and stems as well as for roots. As the latter three fractions were not or only very little higher in the nitrogen-deficient pea leaves and stems, the high concentration of soluble nitrogen must be due to the high content of some other fraction, presumably aminoacids.

Field experiments with dressings of nitrogenous compounds to pea plants. As the pea plants growing on certain soils showed an appearance of nitrogen deficiency, as observed in the above-described experiments with nutrient solutions, field experiments with application of different amounts of nitrogen were carried out. The experimental fields were laid out on clay soils on which the plants showed the above symptoms as well as on soils with a normal plant growth. „Unica" peas were used in most of these experiments. The seed was inoculated with an effective strain of *Rhizobium* before being sown.

In 1943 three experimental fields were laid out on soils on which in previous years the pea growth was inadequate, (field numbers 730, 757 and 770), and two on normal soils (field numbers 755 and 756). The soil of field 770 was a moderate clay that of the other fields was a sandy clay. The pH-value of these soils was about 7, the phosphate and potassium contents were moderate.

A basic dressing of 200 kg of P_2O_5 as superphosphate and 160 kg of K_2O as potassium chloride per ha was given to the fields 755, 756 and

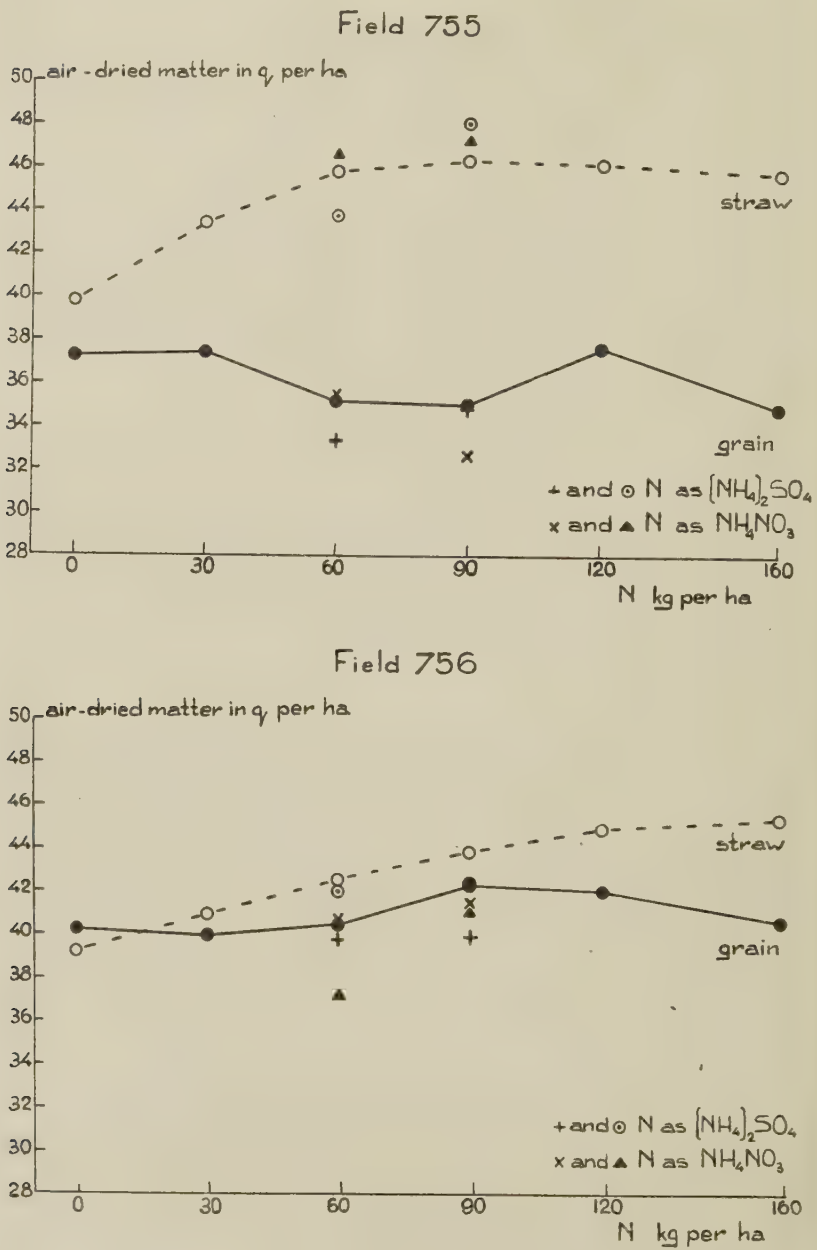

Fig. 5 and 6. Effect of different amounts of calcium nitrate on the yield of peas grown on a clay soil. Some plots were supplied with $(NH_4)_2SO_4$ and NH_4NO_3 respectively (1 q = 100 kg).

757. Field 730 was fertilized with 250 kg P_2O_5 and 125 kg K_2O per ha and field 770 with 100 kg P_2O_5 and 125 kg K_2O. Nitrogen was supplied as ammonium nitrate in applications of 0, 50, 100, 150, 200 and 250 kg of nitrogen per ha on field 730. On the fields 755, 756 and 757 nitrogen was added at a rate of 0, 30, 60, 90, 120 and 160 kg per ha as calcium nitrate. To some plots it was given as ammonium sulphate and ammonium nitrate respectively. Field 770 was dressed with ammonium nitrate at a rate of 50, 100, 150, 200, 250 and 500 kg of N per ha.

As was expected no response to the nitrogenous fertilizers was observed on the fields 755 and 756. The pea plants grew luxuriantly,

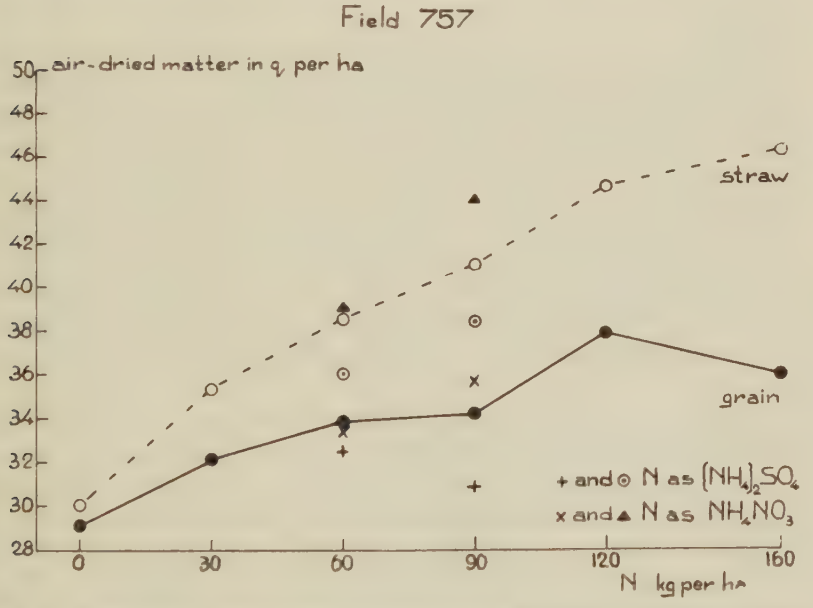

Fig. 7. Effect of different amounts of calcium nitrate on the yield of peas grown on a clay soil. Some plots were supplied with $(NH_4)_2SO_4$ and NH_4NO_3 respectively (1 q = 100 kg).

both those without the application of combined nitrogen as well as those supplied with nitrate, indicating that fixation of gaseous nitrogen by the nodules was fully capable of supplying the plants with nitrogen. As can be seen in fig. 5 and 6 only the yields of straw were somewhat increased by the nitrogen fertilizers *).

On field 757 the pea plants responded clearly to the added nitro-

*) The values plotted in Fig. 5—9 are average yields of two or three plots.

gen. On the nil plots a somewhat poor growth of the plants with a yellow green colour and a dying back of the lower leaves was observed. Supplied with combined nitrogen the peas grew more vigorously with a dark green shade of the leaves. The effect of ammonium sulphate was somewhat inferior to that of calcium nitrate and ammonium nitrate. Not only the yield of straw but that of seed as well was considerably increased by the nitrogen fertilizing (fig. 7). Obviously the fixation of N_2 by the nodules was inadequate to supply the plants with nitrogen.

The question arises to what extent the poor nitrogen fixation of the nodules in this experiment was due to a deficiency of some nutrient element which might have influenced the fixation process, without having affected the growth of the plants when supplied with combined nitrogen. Another possibility is that the formation of the nodules or the fixation of N_2 was suppressed by a parasite such as the larva of *Sitona lineatus* or by a bacteriophage.

Although this point will be dealt with in the following paragraphs, it may already be pointed out that no evidence was obtained that a mineral deficiency was the cause of the poor nitrogen fixation on this field. Applications of phosphate, potassium, boron and molybdenum had no effect on the growth and the appearance of the pea plants.

In a number of cases larvae of *Sitona lineatus* were found to have destroyed many nodules. It is unlikely, however, that these organisms alone were responsible for the poor nitrogen fixation. Although it was believed that bacteriophages played a part in the poor nitrogen fixation, no evidence was obtained that this was true.

Similar results as observed on field 757 were obtained by Miss J. C. Schreuder at this station on some other fields.

Highly significant responses to nitrogen dressings were also found on the fields 730 and 770 (fig. 8 and 9). On these fields, especially on the latter, the pea plants developed very poorly. Investigations carried out by Miss J. C. Schreuder revealed that the plants were infected by a *Fusarium* sp. Fertilizing with nitrogen compounds induced a much better growth of the peas, obviously due to a greater resistance against infection. When the soils were very heavily infected by *Fusarium*, however, only slight improvements in growth were obtained by nitrogen fertilizing (see fig. 9). Miss J. C. Schreuder in cooperation with Dr. F. C. Gerretsen

and the present writer who are studying *Fusarium* diseases of pea plants, will give a more detailed description of these latter experiments in a separate paper.

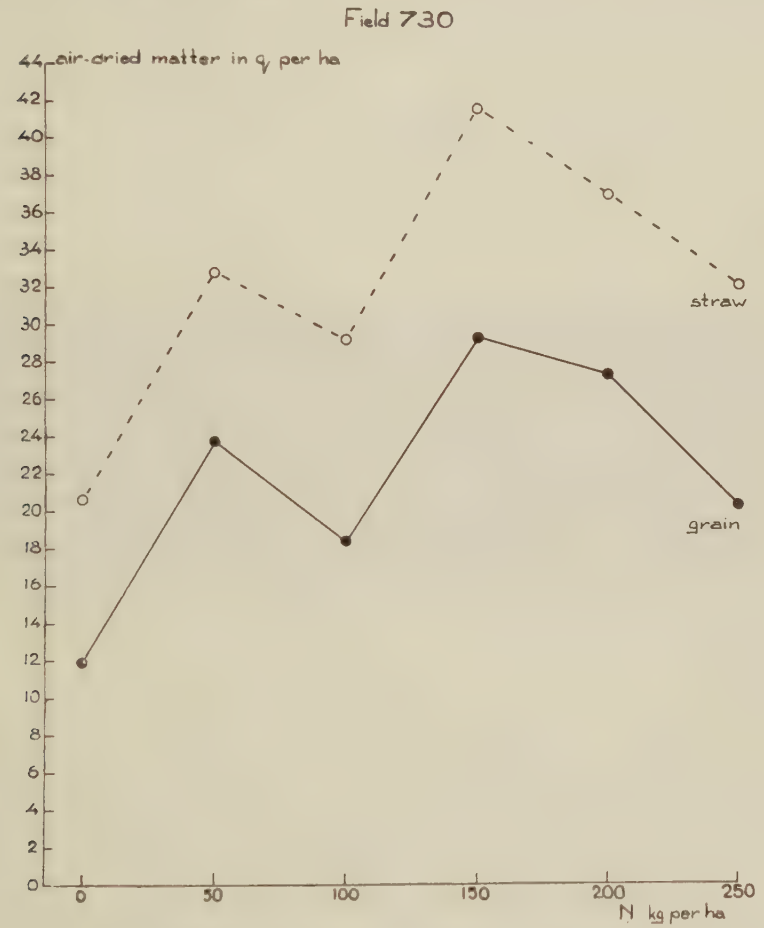

Fig. 8. Effect of different amounts of ammonium nitrate on the yield of peas grown on a clay soil infected by a *Fusarium* sp. (1 q = 100 kg).

The assimilation of different nitrogen compounds by pea plants. In the above-described experiments the nitrogen-deficient pea plants responded much better to nitrate than to ammonium nitrogen (see fig. 2 and 3).

To find out whether pea plants prefer nitrate to ammonium nitrogen an experiment with KNO_3, NH_4NO_3 and $(NH_4)_2SO_4$ as the

sole source of nitrogen was started on May 28, 1942. The nutrient solution used in this experiment contained per litre 0.6 g of $Ca(H_2PO_4)_2$, 0.25 g of Na_2HPO_4 and 0.4 g of K_2SO_4. Mg- and Ca-salts and minor elements were supplied in amounts twice as high as above-mentioned.

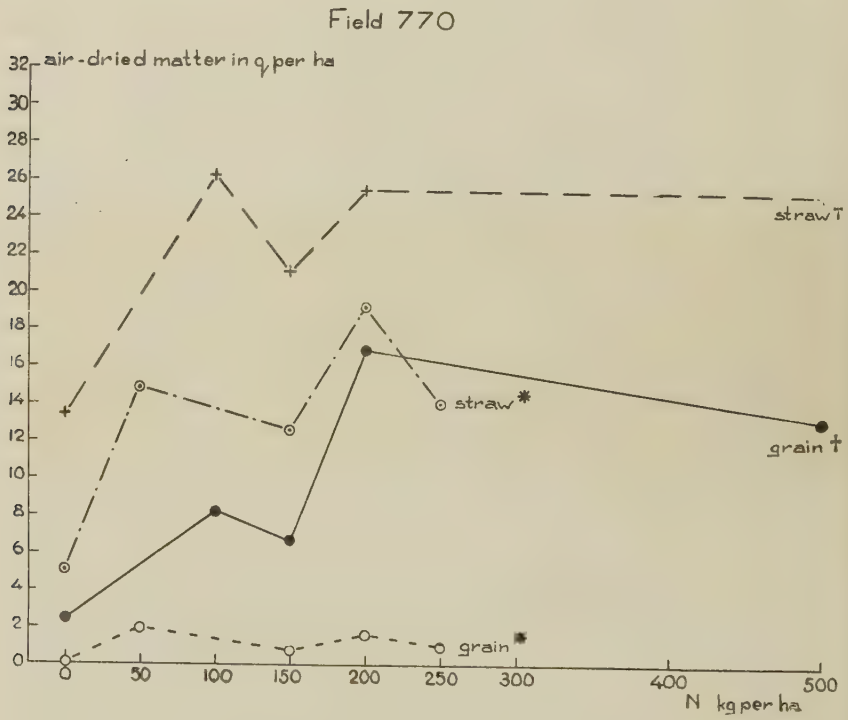

Fig. 9. Effect of different amounts of ammonium nitrate on the yield of peas grown on a clay soil infected by a *Fusarium* sp. *) is a heavily diseased part †), a moderately diseased part of the field. (1 q = 100 kg).

The pH of this solution was daily adjusted to a value of 6.0 with sulphuric acid and a dilute solution of sodium carbonate respectively.

Nitrogen was added to an amount of 25 mg N per jar containing 600 cc of the basic nutrient solution. In these solutions the pea plants with KNO_3 and NH_4NO_3 showed a normal development. Supplied with ammonium sulphate, however, the growth of the plants was very poor with withered lower leaves (see fig. 10). These

withering symptoms commenced with a wilting at the margins of the leaves. As the pH of the nutrient solutions with the three nitrogen compounds was practically the same, i.e. 6.—, it was clear that the poor growth of the plants supplied with ammonium sulphate had something to do with the ammonium salt as such. The possibility exists that under these circumstances pea plants were unable to assimilate ammonium nitrogen. In the following experiment the assimilation of different nitrogen compounds was studied more in detail.

Sterile pea and oat plants were grown in 100 cc Erlenmeyer flasks containing a nitrogen-free nutrient solution.

Fig. 10. Pea plants in nutrient solution without *Rhizobium*; 1 is supplied with $(NH_4)_2SO_4$, 2 with NH_4NO_3 and 3 with KNO_3.

When symptoms of nitrogen deficiency became apparent, 11.6 mg of nitrogen per plant in the form of ammonium sulphate, calcium nitrate and asparagine respectively were added. In addition a phosphate buffer was added to keep the pH as constant as possible.

After some days the plants were harvested and analysed for protein, soluble organic nitrogen, nitrate and ammonia. Similarly to the results of the above-mentioned experiments, the pea plants supplied with ammonium sulphate showed a poor growth with many dead leaves; those with nitrate and asparagine had a much better appearance. During the last days of incubation the asparagine solution of the pea plants became infected by bacteria what resulted in the formation of ammonia **).

**) In a later experiment with sterile cultures a good response of pea plants to asparagine was found.

The oat plants responded similarly to the different nitrogen compounds; the solutions with asparagine remained sterile during the whole incubation period.

The results of this experiment are given in table III.

TABLE III

Assimilation of different nitrogen compounds by pea and oat plants

Plants	Nitrogen compound added	Assimilation period in days	Fresh weight of 6 plants in g	Protein-N of 6 plants in mg	Soluble org. N + NH_4-N of 6 plants in mg	NH_4-N of 6 plants in mg	NO_3-N of 6 plants in mg
Oats	Control, no N	4	1.59	3.48	2.40	0.03	—
,,	$Ca(NO_3)_2$	4	1.46	4.42	5.08	0.12	0.20
,,	$(NH_4)_2SO_4$	4	1.36	4.26	4.01	0.09	0.00
,,	$(NH_4)_2SO_4$ *)	4	1.59	4.19	4.54	0.16	—
,,	Asparagine	4	1.25	4.43	4.20	0.13	—
,,	Control, no N	10	1.26 †)	2.46	2.17	0.11	—
,,	$Ca(NO_3)_2$	10	2.13 †)	7.05	7.37	0.11	—
,,	$(NH_4)_2SO_4$	10	1.82 †)	7.11	7.21	0.21	—
,,	$(NH_4)_2SO_4$ *)	10	1.81 †)	5.40	4.81	0.15	—
Peas	Control, no N	9	10.08	20.84	14.96	0.47	0
,,	$Ca(NO_3)_2$	9	9.68	32.76	17.40	0.36	2.6
,,	$(NH_4)_2SO_4$	9	7.62	27.51	23.50	2.40	0
,,	$(NH_4)_2SO_4$ *)	9	8.76	25.64	17.63	1.18	0
,,	Asparagine	9	8.94	34.74	29.—	0.86	0

*) these plants were not exposed to direct sunshine.

†) 5 plants harvested and analysed.

These data show that in spite of the bad growth of the pea plants supplied with ammonium sulphate, the content of protein and of soluble organic nitrogen had considerably increased, thus indicating that under these circumstances pea plants were able to assimilate the added ammonium nitrogen.

A comparison of the plants supplied with nitrate and ammonium nitrogen shows that in the latter case the ratio protein-N to soluble-N was much lower than with nitrate, whereas a considerable amount of ammonium-N was present. As it is a well-known fact that plants with a non-acid or slightly acid cell sap are unable to accumulate ammonium nitrogen without damage, it is very probable that the high concentration of ammonium nitrogen was the cause of the wilting and necrosis of the pea leaves. The fact that the plants growing at reduced light intensity which, showed less necrotic

leaves, had a considerably lower content of soluble and ammonium nitrogen, is in accordance with the above hypothesis. The plants supplied with asparagine had a high content of soluble N and of protein. Ammonia was relatively low, however, and damage of the leaves was not observed.

The oat plants assimilated the different nitrogen compounds to the same extent; symptoms of damage were not observed.

The question now arises why in the pea plants of this experiment the absorbed ammonium nitrogen was partly accumulated as such and not quantitatively converted into organic nitrogen compounds. As it was expected that the pH of the nutrient medium was an important factor in the assimilation of ammonium nitrogen, an experiment at different pH-values was carried out in 1943.

Assimilation of ammonium nitrogen by pea plants at different pH-levels. In the nutrient solution used in this experiment the concentration of magnesium and calcium sulphate and of minor elements was twice as high as the concentration of the elements above-reported.

For the adjustment of different pH-values the following mixtures of KH_2PO_4 and K_2HPO_4 were used:

pH 5: 272 mg of KH_2PO_4 per jar, containing 600 cc of nutrient solution
pH 6: 218 „ „ „ + 71.3 mg of K_2HPO_4
pH 7: 82 „ „ „ + 249 „ „ „
pH 8: 356 „ „ „

The potassium content of every jar was brought up to the same level with potassium sulphate.

"Unica" peas were transplanted into the glass jars on July 20, every jar containing three plants. On July 31 5 mg of nitrogen as ammonium sulphate was added to four cultures of every pH range.

On August 3 again 10 mg of ammonium nitrogen was given and on August 7 again 25 mg. One set of cultures was supplied with potassium nitrate.

At the latter date clear differences were shown. At pH 5 the plants supplied with ammonium sulphate were poorly developed with many dead lower leaves. Of the higher leaves the margins were bent down. Supplied with nitrate these symptoms did not occur and the plants made a good growth.

At pH 6 an only slightly better appearance of the NH_4-plants was observed than at pH 5. At pH 7 and 8, however, a normal development occurred without any damage of the leaves. This result clearly shows that the assimilation of ammonium nitrogen by pea plants depends on the pH of the nutrient solution. Only in media with a neutral or alkaline reaction these plants are able to use ammonium sulphate as a source of nitrogen. In media with a pH of 6 or lower, however, this compound is very toxic. It is very probable that the contradictory results of various authors in respect of the nitrogen nutrition of leguminous plants (P a r d o) [7]) are due to a different pH of the nutrient medium used.

After being supplied with another 50 mg of nitrogen per jar as ammonium sulphate and potassium nitrate respectively on August 21, the plants of this experiment were harvested on August 25. At this date a great part of the leaves of the NH_4-plants growing at pH 5 was wilted. At pH 6 also a considerable damage of the leaves was observed. At pH 7 and 8 the plants grew quite normally without any damage of the leaves.

The yields of the plants (fresh weights) are recorded in table IV.

TABLE IV

Effect of ammonium sulphate and potassium nitrate on the yield of peas grown in nutrient solutions at different pH

pH of nutrient solution	Nitrogen compound supplied	Leaves fresh weight in g	Stems fresh weight in g	Roots fresh weight in g
5	$(NH_4)_2SO_4$	0.91	1.55	4.—
5	KNO_3	3.19	3.56	4.97
6	$(NH_4)_2SO_4$	2.10	1.99	4.93
6	KNO_3	3.80	2.94	6.83
7	$(NH_4)_2SO_4$	5.93	4.35	7.16
7	KNO_3	6.—	4.77	8.56
8	$(NH_4)_2SO_4$	5.36	3.85	6.13
8	KNO_3	6.55	5.67	10.42

These results clearly show the essentiality of a non-acid reaction of the nutrient solution for the development of pea plants supplied with ammonium nitrogen. Although the NO_3-plants gave higher yields at pH 7 and 8 than at lower values, they made a normal growth in the latter case, without necrotic leaves.

The fresh tissues of these plants were analysed for ammonium nitrogen by adding a borate buffer and distilling the ammonia

under reduced pressure at 40°C. The results of the analyses are recorded in table V.

TABLE V

Ammonium nitrogen in pea tissues grown at different pH-values

pH of nutrient solution	Nitrogen compound supplied	mg NH_4–N per 10 g of fresh tissue *)			
		Leaves		Stems	Roots
		Wilted but not necrotic tissue	Normal tissue		
5	$(NH_4)_2SO_4$	10.94	—	1.75	0.23
5	KNO_3	0.40	0.05	0.10	0.04
6	$(NH_4)_2SO_4$	5.96	1.54	1.40	0.20
6	KNO_3	—	0.00	0.02	0.07
7	$(NH_4)_2SO_4$	—	0.10	0.16	0.12
7	KNO_3	—	0.04	0.22	0.22
8	$(NH_4)_2SO_4$	—	0.05	0.05	0.28
8	KNO_3	—	0.00	0.01	0.18

*) average values of two determinations.

These data reveal a close connection between the occurrence of wilted leaves and the content of ammonium nitrogen. The higher the pH of the nutrient medium the lower this content. Thus it seems very probable that a ready assimilation of ammonium nitrogen by pea plants can take place only when the pH of the nutrient solution is 7 or higher. In the following paragraph the influence of the pH on the ammonia assimilation will be dealt with more in detail. As in the above-mentioned experiment the nutrient solutions were not kept completely sterile, the following one was carried out with sterile cultures.

Assimilation of ammonium and nitrate nitrogen by sterile pea plants. In this experiment 1 l Erlenmeyer flasks were used as culture vessels. They were provided with rubber stoppers containing three tubes, one holding the plant, one for aerating the nutrient solution and a third for taking samples and adjusting the pH.

Germinated sterile peas were placed in the tubes which according to Gerretsen[3]) were filled with paraffined cork filings containing a silver preparation (katadyne bolus, diatomaceous earth, covered with porous silver), which prevents the intrusion of moulds and bacteria into the nutrient medium, while the plants are not damaged.

The nutrient solution used in this experiment, had about the same composition as in the previous experiment, different mixtures of KH_2PO_4 and K_2HPO_4 being used to obtain pH-values of 5.2, 6.0, 7.0 and 7.6 respectively. 50 mg of nitrogen as KNO_3, $(NH_4)_2SO_4$ and $(NH_4)_2HPO_4$ respectively were supplied on September 28. Some plants remained without nitrogen. As in the other experiments the pH-values were carefully maintained at the above values by adjusting with diluted sulphuric acid and a solution of sodium carbonate respectively.

The results of this experiment agreed well with those of the preceding one. At pH 5.2 the growth of the plants with $(NH_4)_2SO_4$ and $(NH_4)_2HPO_4$ was very poor with a wilting and afterwards a necrosis of the leaves. Supplied with nitrate normal leaves developed. At pH 6 the plants made a considerably better growth but many leaves still showed the symptoms of ammonium injury. At pH 7 and 7.6 normal plants were obtained. The fresh weights of the plants of this experiment are given in table VI. It should be stressed that these plants were harvested at different dates. The data of this table agree well with those of the previous experiment.

TABLE VI

Fresh weights of pea plants grown in sterile culture solutions at different pH-values

Nitrogen compound supplied	Date of harvesting	pH = 5.2		pH = 6		pH = 7		pH = 7.6	
		Leaves + stems in g	Roots in g	Leaves + stems in g	Roots in g	Leaves + stems in g	Roots in g	Leaves + stems in g	Roots in g
no nitrogen	Oct. 21	0.50	1.60	0.80	3.30	1.00	4.34	0.91	3.70
,, ,,	,, 25	0.52	2.30	0.78	4.55	0.47	2.06	0.49	2.81
KNO_3	,, 20	1.78	3.48	1.45	3.39	2.53	4.60	1.14	2.31
,,	,, 25	1.89	3.40	1.97	3.53	4.77	7.60	2.97	5.62
$(NH_4)_2SO_4$	,, 15	0.83	2.30	1.38	4.28	1.86	3.53	1.56	3.55
,,	,, 22	0.80	1.93	1.12	3.06	2.00	4.40	3.05	5.30
,,	,, 23	0.53	1.30	1.40	3.77	2.94	7.50	3.80	6.70
$(NH_4)_2HPO_4$	,, 18	0.95	3.08	1.32	2.83	2.16	5.47	2.28	4.53
,,	,, 22	0.83	2.32	0.86	2.40	—	—	2.35	4.31
,,	,, 22	1.14	2.84	0.96	2.25	1.85	3.05	3.55	7.62

In order to find out what might be the cause of the ammonia injury of the NH_4-plants growing in acid media, starch tests were

carried out in leaves showing a different rate of damage. The leaves were boiled in alcohol to remove the chlorophyll and then treated with a solution of J_2 in KJ. The rate of blackening of the leaves being a measure of the starch content, estimates of this compound were thus made in a simple way. Table VII contains the results of these estimates.

TABLE VII

Influence of the pH of the nutrient medium and the nitrogen compound supplied on the starch content of peas growing in sterile solutions (Yields in table VI)

pH of nutrient solution	Nitrogen compound supplied	Appearance of the leaves investigated *)	Starch test
5.2	no nitrogen	young leaves, pale green, margins somewhat necrotic	negative
5.2	KNO_3	young leaves, no damage	highly positive
5.2	$(NH_4)_2SO_4$	young, light green leaves with necrotic margins	negative
5.2	$(NH_4)_2SO_4$	normal, without damage	moderately positive
5.2	$(NH_4)_2HPO_4$	heavy symptoms of ammonium injury	negative
5.2	$(NH_4)_2HPO_4$	normal, somewhat light green	slightly positive
6	no nitrogen	young, pale green leaves, no damage	moderately positive
6	KNO_3	young, normal leaves	highly positive
6	$(NH_4)_2SO_4$	damaged	negative
6	$(NH_4)_2SO_4$	normal	moderately positive
6	$(NH_4)_2HPO_4$	damaged	negative
6	$(NH_4)_2HPO_4$	normal	moderately positive
7	no nitrogen	pale green, slightly damaged	positive
7	KNO_3	normal	highly positive
7	$(NH_4)_2SO_4$	normal	,,
7	$(NH_4)_2HPO_4$	normal	,,
7.6	no nitrogen	pale green, slightly necrotic	positive
7.6	KNO_3	normal green	highly positive
7.6	$(NH_4)_2SO_4$	normal green	,,
7.6	$(NH_4)_2HPO_4$	normal green	,,

*) 4–6 leaves of every sample were tested.

These data show that the poor growth of the ammonium-supplied plants and the rate of damage of the leaves are correlated with the starch content. Heavily damaged leaves are practically free from starch. The higher the pH of the nutrient medium the higher the starch content of the leaves.

The fact that the starch content of the plants growing in a ni-

trogen-free solution also depends on the pH of the nutrient solution, shows that it is the hydrogen ion concentration rather than the ammonium salt which influences the formation of starch. Since the assimilation of ammonium nitrogen requires the presence of carbohydrates to convert the absorbed ammonia into organic nitrogen compounds, it is probable that the poor assimilation of ammonium nitrogen by pea plants growing in acid nutrient media is due to the low carbohydrate level in the plant. As a result ammonia is accumulating to a concentration which is toxic to the cells.

To answer the question in what way the pH of the nutrient medium may influence the starch production in the leaves, pH determinations were carried out in leaf and root tissues of the above plants. The fresh plant tissues were ground in a mortar and in the pulp the pH was determined by a quinhydrone electrode. The results of these determinations, given in table VIII, show that the hydrogen

TABLE VIII

Influence of the pH of the nutrient solution on the pH of tissues of peas grown in nutrient solutions at different pH-values

Nitrogen compound supplied	Leaves and stems				Roots			
	pH=5.2	pH=6	pH=7	pH=7.6	pH=5.2	pH=6	pH=7	pH=7.6
no nitrogen	5.89	5.93	6.07	6.17	5.56	5.61	5.73	5.83
,, ,,	(5.65)*)	(5.96)	(6.09)	(6.00)				
,, ,,	5.40	5.80	5.92	5.94	5.48	5.55	5.70	5.90
,, ,,	(5.40)		(5.96)	(6.02)				
KNO_3	6.18	6.20	6.24	6.24	5.86	5.96	6.04	6.24
,,	6.10	6.13	6.40	6.32	5.69	5.80	5.91	6.12
$(NH_4)_2SO_4$	5.69	5.80	6.30	6.40	5.54	5.62	5.82	6.12
,,	(5.21)	(5.67)						
,,	5.90	5.82	6.20	6.36	5.58	5.53	5.68	5.93
,,	(5.43)	(5.50)						
$(NH_4)_2HPO_4$	5.86	6.04	6.33	6.48	5.80	5.88	6.00	6.16
,,	5.73	5.70	6.13	6.30	5.54	5.70	5.72	5.90
,,	(5.52)	(5.50)						

*) ( ): necrotic tissue.

ion concentration of the leaf plus stem tissue is influenced by the pH of the nutrient medium and by the nitrogen compound absorbed. In solutions containing ammonium sulphate or ammonium phosphate in which the hydrogen ion concentration varied from pH 5.2–7.6, the pH of leaf plus stem varied from 5.70 (5.20 in necrotic tissues) to 6.40. Plants supplied with KNO_3 although grown in a

nutrient solution of the same hydrogen ion concentration as those with ammonium sulphate had a considerably higher pH in the leaf plus stem tissues. The differences between ammonium and nitrate plants only existed in the acid media; in the neutral or alkaline solutions they were negligible.

These results are in contrast with those obtained in similar experiments with tomato and potato plants. The tomatoes were grown in nutrient solutions at the same range of pH-values as in the above experiment with peas. Nitrogen was supplied as ammonium sulphate and potassium nitrate respectively. No influence of the nutrient media on the hydrogen ion concentration of the leaf and stem tissues was exerted, however, a pH of 5.8 being found at all the pH levels and with both nitrogen compounds. In the ground root tissues the pH-values ranged from 5.6 in the acid media to 5.8 in the neutral and alkaline solutions.

In the case of potatoes the pH of the soil in which the plants had grown varied from 4.2 7.0 The pH of the ground potato tubers only ranged from 5.80–5.95. The results of the experiments with tomato and potato plants are in good agreement with those reported by Hoagland and Davis[4]) and by Mevius[6]).

From the results of the experiments described in this paragraph it may be concluded that pea plants growing in acid nutrient media without combined nitrogen or supplied with ammonium phosphate or ammonium sulphate have a considerably higher hydrogen ion concentration in their leaf and stem tissues than those growing in neutral and alkaline solutions. Due to (or perhaps parallel with) this low pH the starch content of the leaves is low, and the assimilation of ammonia which requires a high carbohydrate level in the plant, is inadequate. Accumulation of ammonia takes place, followed by wilting and necrosis of the leaves and a poor growth of the whole plant.

The effect of zinc and boron on the fixation of nitrogen by the nodules of pea plants. The question whether or not a certain element is essential for the production of nodules or for the fixation of nitrogen, is not easy to solve. It can be done by growing the leguminous plants with increasing amounts of the element concerned, both without and with combined nitrogen. In the former case the nodules have to supply the plants with nitrogen. When the element has a specific

function in the fixation of nitrogen, differences between the two sets of plants may be expected due to a different nitrogen supply. However, when no differences can be observed, it is not sure that the element in question is not essential for the formation of nodules or the fixation of nitrogen. It only means that the requirements of the nodules are less than those of the other parts of the plant so that nitrogen fixation can take place. An exact observation of the habit and growth of the plants as well as of the nodules, in combination with nitrogen determinations carried out at the right moment may in this respect provide more information. This is particularly of importance in the case of elements in the absence of which deficiency symptoms occur, quite different from those of nitrogen deficiency.

In the following paragraphs some experiments will be described dealing with the influence of zinc and boron on the growth and nitrogen fixation of pea plants.

The importance of zinc in the nitrogen fixation by peas. To find out whether or not zinc is an important element in the fixation of nitrogen by the nodules of pea plants, culture solution experiments were carried out in 1943. In this experiment a nutrient solution of the following composition was used:

Water, distilled in a glass apparatus, 1 l

KH_2PO_4	0.279 g	$FeCl_3.6H_2O$	15 mg
K_2HPO_4	0.193 g	$MnSO_4.4H_2O$	0.6 mg
KNO_3	0.200 g	$CuSO_4.5H_2O$	0.15 mg
$MgSO_4.7H_2O$	0.108 g	H_3BO_3	0.15 mg
$CaSO_4.2H_2O$	0.225 g	Na_2MoO_4	0.06 mg

"Weck" glass jars of 1.5 l capacity were used as containers. In every jar three germinated "Unica" peas were planted on March 27. The root system as well as the nutrient medium were inoculated with an effective strain of *Rhizobium*.

In the first days of May symptoms of zinc deficiency became apparent. The margins of the lowest leaves became necrotic, taking a gray white colour. Some days later the whole leaves had died. This process advanced upwards so that in the middle of May only the youngest leaves were still alive. The poorly growing plants were able,

however, to develop flowers and pods with some seeds. In contrast to other trace elements as for instance copper and boron, zinc apparently plays no part in the emerging of young leaves and in the seed formation.

The zinc-deficient plants developed many root nodules. Although these nodules were of a small size, probably due to the necrosis of a great deal of the plants, they fixed nitrogen from the atmosphere normally, as was deduced from the dark green colour of the living leaves, and from the fact that plants without combined nitrogen *) showed the same appearance as those supplied with 200 mg of nitrogen as KNO_3 per pot.

The yields of the plants of this experiment are given in table IX.

TABLE IX

Effect of zinc on the yield of pea plants

$ZnSO_4.7H_2O$	N as KNO_3	Yields, dry weight in g		
		Seeds *)	Straw	Root
0	28	1.26	2.14	1.—
0	200	0.56	2.52	1.35
0.5 mg	28	— †)	7.44	2.18
0.5 mg	200	9.54	7.69	1.52

*) average yields of two and three pots respectively.

†) the plants of these pots were harvested before the seeds had developed.

The importance of boron in the formation of nodules and the fixation of nitrogen. In a preliminary experiment with pea plants in nutrient solutions, carried out in 1941, no development of nodules was observed in the absence of boron, notwithstanding that the culture solution as well as the root system were inoculated with an effective strain of *Rhizobium*. The fact that in this solution the secondary roots of the plants were very short, makes it highly probable that it is the plant rather than the bacteria which is affected by the lack of boron. This is in agreement with the fact that *Rhizobium*, similar to other bacteria, does not require boron, whereas pea plants need considerable amounts of it.

Due to the lack of nodules the nitrogen supply of the boron-deficient plants became inadequate; light green leaves appeared

*) the very small amount of nitrate nitrogen initially supplied, was used up in the first stage of growth.

and some weeks later the plants died from nitrogen deficiency. The typical symptoms of boron deficiency, which particularly can be seen in the young leaves and buds of the plant and which are quite different from the symptoms of nitrogen deficiency, were not observed.

Supplied with 0.5 mg of boric acid normal nodulation and nitrogen fixation occurred (table X).

TABLE X

Effect of boron on the nitrogen fixation of pea plants			
H_3BO_3 mg per pot	Yields, dry weight in g		N in total plants in mg §)
	Tops	Roots	
0	1.10 *)	0.93	40.7
0.5	3.10 †)	1.04	110.8

*) average values of two cultures.

†) average values of four cultures.

§) the nutrient solution was initially supplied with a very small amount of combined nitrogen.

The results of this experiment are in full agreement with those of Brenchley and Thornton[2]) who in 1925 showed that boron is essential for the development of nodules on roots of *Vicia faba*.

In order to find out to what extent an application of combined nitrogen may decrease the boron requirement of pea plants, an experiment with increasing amounts of boric acid at two different nitrogen levels was carried out in 1942. "Unica" pea plants were transferred to glass jars containing 1.8 l of a nutrient solution on April 23.

This solution had the same composition as above-mentioned except that phosphate was supplied in a concentration of 180 mg of KH_2PO_4 and 60 mg of K_2HPO_4 per l.

Boric acid was added in amounts of 0,10, 20, 150 and 1650 *) γ per culture. One set of cultures was supplied with 130 mg of nitrogen per culture in the form of KNO_3 and 30 mg N as $(NH_4)_2SO_4$, the other one received only 10 mg of nitrogen as KNO_3 and 5 mg as $(NH_4)_2SO_4$. As in the preceding experiments the roots of the plants and the nutrient solution of all the cultures were inoculated with an effective strain of *Rhizobium*.

After four weeks growth pronounced differences were observed.

*) the largest amounts were added in three or four parts.

In the absence of combined nitrogen *) the plants without boron and those supplied with 10 γ of boric acid developed only very small, light yellow, somewhat slimy nodules which apparently were unable to fix nitrogen. The leaves and stems of these plants showed pro-

Fig. 11

Fig. 12

Fig. 11 and 12. Pea plants in nutrient solution with different amounts of boric acid (1: without B, 2 : 10, 3 : 20, 4 : 150 and 5 : 1650 γ of H_3BO_3 per pot).

Fig. 11: nitrogen supplied by the nodules. Fig. 12: plants supplied with combined nitrogen. The plants of the pots 1 and 2 in fig. 11 show pronounced symptoms of nitrogen deficiency, those of 3 and 4 in fig. 11 and of 1, 2, 3 and 4 of fig. 12 show symptoms of boron deficiency.

*) the very small amount of combined nitrogen initially supplied to these cultures was used up in the first stage of growth.

nounced symptoms of nitrogen deficiency, but not the characteristic symptoms of boron deficiency. Some weeks later these plants died from nitrogen starvation.

The plants supplied with 20 γ of boric acid had many well-developed nodules on their roots and consequently they were well-supplied with nitrogen. The leaves were dark green and until June 1 the growth of these plants proceeded quite normally. At that date, however, symptoms of boron deficiency became apparent. The youngest leaves showed yellow margins while their tops often bent upwards and became necrotic. If this happened when the leaves were very young no further growth took place and usually the whole growing-point died off. Secondary buds developed and this resulted in a bushy character of the whole plant (see fig. 11 and 12).

Supplied with 150 γ of boric acid only light deficiency symptoms were seen in the youngest leaves. Some growing-points died, but in other cases normal blooming and fruit setting took place. With the highest amount of H_3BO_3 no deficiency symptoms were observed.

The plants supplied with combined nitrogen did not respond less to boron than those depending on the nitrogen fixation of the nodules. Only in the absence of boron and with the lowest amount of boric acid did these plants make initially a better growth, due to the fact that they did not suffer from nitrogen deficiency. Pronounced symptoms of boron deficiency became apparent, however, at an early date and as a result the plants developed very poorly. In contrast to the plants without combined nitrogen they remained alive for a considerable length of time.

With application of 20 γ and particularly with 150 γ of H_3BO_3 the symptoms of boron deficiency became apparent at an earlier date and more seriously than was the case with plants depending on the nitrogen fixation of the nodules. Obviously this was due to a better supply of nitrogen, which enabled the plants to make a somewhat quicker growth with a higher production of dry matter, and as a result a higher consumption of boron.

Although the plants supplied with combined nitrogen gave higher yields of dry matter than those deriving their nitrogen from the nodules the general trend of both yield curves is about the same (fig. 13).

The results of this experiment have shown that the boron requirement of the growing leaves and stems in general is considerably

higher than that of the nodules. Apparently a small amount of boron initially available in the culture solution is high enough to develop the nodules and once being formed nitrogen fixation proceeds normally, independent of the occurrence of pronounced symptoms of boron deficiency in the tops. Only when boron is totally absent in the nutrient solution when the nodules are developing, is the nodule-forming tissue more affected by the lack of this element than the tops. As a result normal nodules are not developed and the plants die from nitrogen deficiency.

Experiment with boron-deficient sand-peat

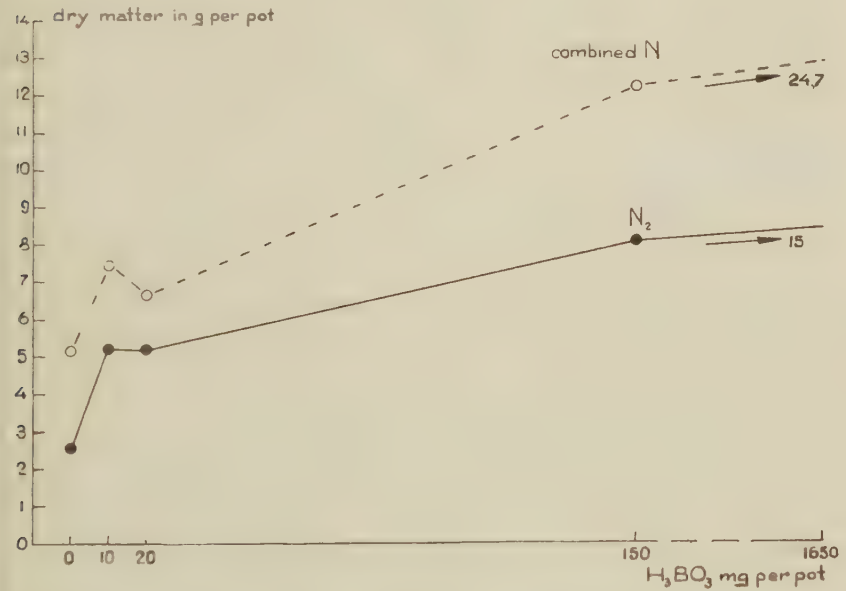

Fig. 13. Effect of different amounts of boron on the yield of pea plants grown in nutrient solution, inoculated with *Rhizobium*, with a very small and a normal supply of combined nitrogen respectively.

mixtures. In order to elucidate the behaviour of pea plants towards boron when growing in soils poor in this element, experiments with a sand-peat mixture were carried out.

The quarz sand used in this experiment was treated with 20% nitric acid and then washed free from acid with distilled water. The peat was in the same way treated with 5% sulphuric acid.

Six parts of sand were mixed with one part of peat. To this mix-

ture calcium carbonate was added to attain a pH-value of 6.2. Glass jars containing 2 kg of the mixture were used as culture vessels. Every pot was dressed with the following salts:

$Ca(H_2PO_4)_2$	1.— g	$FeCl_3.6H_2O$ . . .	25.— mg
K_2SO_4	0.5 g	$CuSO_4.5H_2O$ . . .	10.— mg
(1.5 g when the small amount of nitrogen was added)		$MnSO_4.4H_2O$ . .	10.— mg
$MgSO_4.7H_2O$	0.5 g	$ZnSO_4.7H_2O$ . . .	5.— mg
KNO_3	0.2 g	$Na_2MoO_4.2H_2O$ . .	2.— mg

As usual the experiment consisted of two sets of pots, one with only a small amount of nitrogen (0.2 g KNO_3 per pot) and one with 2.— g of KNO_3. Boron was supplied in amounts of: 0, 0.05, 0.1, 0.2, 0.3, 0.4, 0.5, 1.2, 3, 4, 5 and 10 mg of boric acid per pot. Germinated "Unica" peas inoculated with a suspension of *Rhizobium* were planted on March 23, 1943.

Very pronounced symptoms of boron deficiency were seen at an early date in the pea plants, not only in those where boron was absent but also in the plants supplied with 50, 100, 200 and 300 γ of H_3BO_3. An application of 0.5 mg of boric acid gave a considerably better development but with higher amounts of H_3BO_3 much higher yields were obtained (fig. 14). These results suggest that considerable amounts of boron were fixed by the peat.

In contrast to the above experiment with nutrient solutions, symptoms of nitrogen deficiency were not observed in the boron-deficient plants, notwithstanding the fact that the nodulation of these plants was very poor. Obviously the small amount of potassium nitrate gave an adequate supply of nitrogen to the poorly growing plants

A remarkable difference was found between the two sets of plants. Those supplied with 2.0 g of potassium nitrate suffered much more from B-deficiency than the corresponding ones with 0.2 g (fig. 14). This different behaviour was still more clearly shown when pea plants were grown for the second time in the above pots in which, in the first experiment, plant growth totally failed. After the poorly developed plants of these pots had been removed, additional amounts of H_3BO_3 were given and on May 14 peas were planted for the second time. The yields of this second experiment are given in table XI. In the plants without nitrate, boron deficiency was only observed at a rate of 300 and 400 γ of H_3BO_3 per pot, while

supplied with nitrate even the plants with the highest amounts of boric acid were still slightly deficient in boron.

TABLE XI

Effect of boron on the yields of pea plants grown in sand-peat mixtures

H_3BO_3	Without combined nitrogen			Supplied with KNO_3		
γ per pot	Grain in g	Straw in g	pH of soil	Grain in g	Straw in g	pH of soil
300	3.02	11.12	6.75	—	6.30	6.90
400	2.—	8.—	6.65	0.38	7.68	6.95
500	7.25	8.85	6.70	2.35	8.02	6.95
600	7.22	8.65	6.90	1.68	8.85	6.85
650	11.47	12.32	6.65	0.13	6.45	6.95
700	10.95	12.10	6.65	1.82	11.—	7.—
750	9.25	8.98	6.95	1.82	9.83	7.—
900	8.10	10.37	6.70	3.—	8.33	7.10
1000	9.20	11.05	6.70	5.77	9.75	6.95

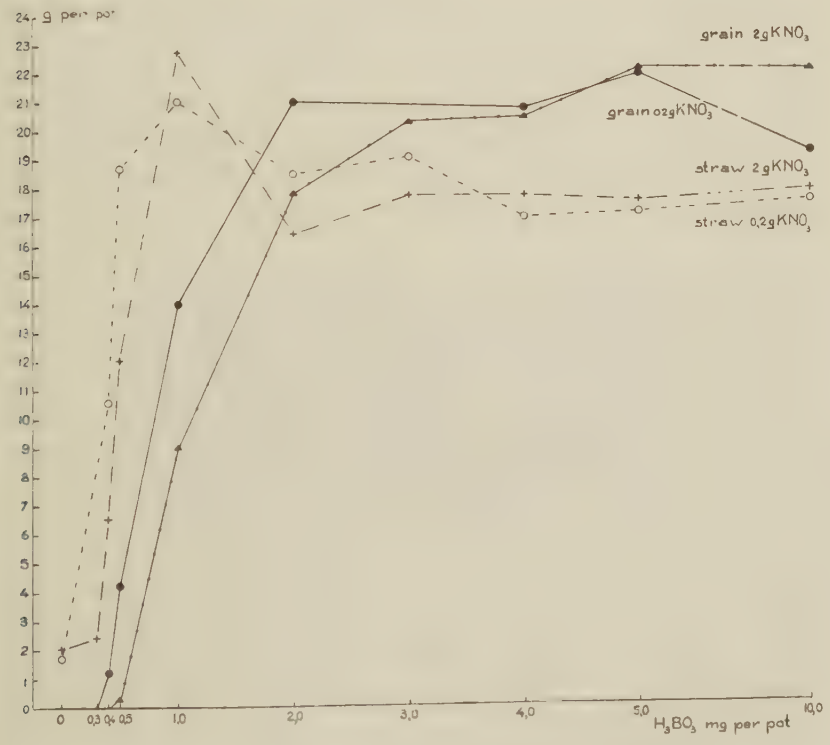

Fig. 14. Effect of different amounts of boron on the yield of pea plants grown in a sand-peat mixture, inoculated with *Rhizobium*, with a very small and a normal supply of nitrate respectively.

As it was suggested *) that the higher requirement of boron of the nitrate plants was due to a more alkaline reaction of the nutrient medium following the absorption of the nitrate, pH-values of the soil were measured after harvesting the plants.

As is seen from table XI the sand-peat mixtures to which nitrate was added had consistently higher pH-values. It may be expected that in the rhizosphere, from which the roots absorb the nutrients, the differences would have been more evident.

Experiments with soil. In the autumn of 1941 a pot experiment was carried out with 24 different soils from fields in the southern part of the Netherlands on which boron deficiency in beets had been observed, and from fields in the province of Groningen on which the growth of peas was inadequate.

Eight glass jars containing about 2.5 kg of soil per jar were filled with each soil. Per pot the following basic dressing was added: 0.5 g of K_2SO_4, 0.5 g of $Ca(H_2PO_4)_2$ and 50 mg of $MnSO_4$. 2 pots remained without further dressing, 2 were supplied with 1.25 g of NH_4NO_3 per pot, 2 with 20 mg of boric acid and 2 with 20 mg of H_3BO_3 and in addition 1.25 g of NH_4NO_3. Each soil was inoculated with 5 cc of a suspension of *Rhizobium*. "Mansholt" peas were planted on August 27, 1941.

Due to the warm weather in September and October 1941 a fairly good growth of the pea plants took place. Although in many cases a beneficial effect of the combined nitrogen was observed, a response to boron was only in a few cases perceptible. On one sandy soil this was particularly true (see fig. 15). Without application of boron the nodules were small with an abnormal pale white yellow and sometimes black brown colour. Supplied with boric acid normal nodules with a reddish brown colour developed.

The experiment was repeated in the spring of 1942 with five soils, on which the plants more or less had responded to boron. As in the preceding year glass jars were used containing about 2.5 kg of soil. As a basic dressing the following salts were added per pot: 0.5 g K_2SO_4, 0.5 g $Ca(H_2PO_4)_2$, 0.2 g $MgSO_4.7H_2O$. The plan of this experiment was as follows:

a) no boron, 62.5 mg of ammonium nitrate.

b) 20 mg of H_3BO_3, 62.5 mg of ammonium nitrate.

*) see Lehr (5).

c) no boron, 1250 mg of ammonium nitrate.

d) 20 mg of H_3BO_3, 1250 mg of ammonium nitrate.

On two soils, a sandy and a clay soil, the plants responded to boron. Deficiency symptoms became only apparent, however, after the plants had already grown for nearly two months. The growth of the plants slackened and the leaves showed a light green and later a yellow colour. They ripened at an earlier stage than those supplied with either boron or nitrogen and gave lower yields (see table XII). These symptoms resembled those of nitrogen deficiency.

TABLE XII

Effect of boron on the yields *) of pea plants grown in soil

Soil	62.5 mg of NH_4NO_3 per pot				1250 mg of NH_4NO_3 per pot			
	No boron		20 mg of H_3BO_3 per pot		No boron		20 mg of H_3BO_3 per pot	
	Grain in g	Straw in g	Grain in g	Straw in g	Grain in g	Straw in g	Grain in g	Straw i g
Clay soil	5.25	4.60	7.45	6.92	4.20†)	6.17	10.05	8.82
,, ,,	5.60	4.60	8.05	6.40	7.13	7.94	8,62	8.45
Sandy soil	4.97	4.45	9.22	9.17	5.89†)	6.12	9.19	7.46
,, ,,	4.71	4.94	10.30	7.10	13.—	11.12	2.55†)	5.16

*) dry weight.

†) the low yield of these pots was due to an irregular germination of the seeds.

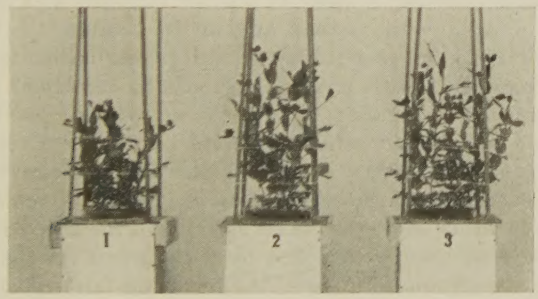

Fig. 15. Effect of boron and combined nitrogen on the development of pea plants growing in a sandy soil. 1: without B and combined N, 2: 20 mg of H_3BO_3 per pot (no N), 3: 1.25 g of NH_4NO_3 per pot (no B).

Although many nodules were present, their colour was black and they were shriveled at the time of harvesting. Supplied with 20 mg of H_3BO_3 normal nodules occurred. When NH_4NO_3 was applied, practically no nodules had been developed.

The most important result of this experiment was, however, that no typical symptoms of boron deficiency were observed in the tops of the plants growing without application of boric acid. The blooming and fruit setting were quite normal and the lower yields of seeds and straw were only due to the early ripening of the plants owing to an inadequate fixation of nitrogen by the nodules. It is not clear why in these soils the boron requirement of the nodules seems higher than that of the tops, while in the experiment with nutrient solutions in general an opposite result was found. It may be that the development of nodules in the soil started relatively late when most of the available boron already had been absorbed by the plants.

Summary

The effect of different amounts of ammonium nitrate on the development of pea plants growing in nutrient solutions in the absence of *Rhizobium* was investigated. It was found that these plants behaved to nitrogen quite differently from oat plants which were grown in a similar set of culture solutions. Nitrogen-deficient pea plants became yellow and died some weeks later. This was in contrast to oat plants which were able to complete their life cycle in a solution free from nitrogen.

Nitrogen-deficient oat plants responded much more readily to newly introduced nitrogen than pea plants.

Tops of pea plants with pronounced symptoms of nitrogen deficiency had a considerably higher content of soluble nitrogen and less protein-N than plants supplied with a small amount of combined nitrogen. With higher applications of nitrogen protein as well as soluble nitrogen increased.

In field experiments with peas no response to combined nitrogen was observed on soils on which always a good growth of these plants took place. On soils with a poor pea growth in previous years a clear response to the supplied nitrogen was observed. In one case the inadequate fixation of nitrogen was partly due to the larvea of *Sitona lineatus* which destroyed the nodules. On two other fields the plants growing in the absence of combined nitrogen were more heavily attacked by a *Fusarium* sp. than those well-supplied with nitrogenous fertilizers. As a result the latter plants gave much higher yields.

In nutrient solutions of pH 6 the effect of some nitrogenous compounds on the growth of pea plants was investigated. Ammonium nitrate and potassium nitrate provided a much superior source of nitrogen than ammonium sulphate. Plants supplied with the latter compound developed poorly and had many wilted and necrotic leaves. It was shown that the poor growth of these plants was not due to their inability to assimilate ammonium nitrogen. The ratio soluble N to protein N was much higher in the plants supplied with $(NH_4)_2SO_4$ than in the nitrate plants. In addition the concentration of ammonium nitrogen in the former plants was high.

In culture solutions of different hydrogen ion concentrations a ready assimilation of ammonium sulphate by pea plants without necrosis of the leaves was found at pH-values of 7 and higher. The ammonia content was very low in these plants, while at pH 6 and 5 many necrotic leaves were observed and the NH_4-content of the leaves and stems was considerably higher.

In a similar experiment with sterile plants it was found that the leaves of pea plants growing at pH 5.2 and 6, and supplied with ammonium sulphate or ammonium phosphate had a very low content of starch. In the wilted leaves practically no starch was found. The pH-values of the ground leaves and stems of these plants were considerably lower than those of plants supplied with KNO_3 or with $(NH_4)_2SO_4$ in neutral or weakly alkaline solutions, which had a much higher starch content in their leaves. It was concluded that the necrosis of the leaves and the poor growth of the pea plants supplied with $(NH_4)_2SO_4$ at pH-values of 6 and lower were due to an injury by ammonia, which accumulated owing to an inadequate assimilation of this compound in leaf tissues without starch and with a relatively high hydrogen ion concentration.

Pea plants growing in a zinc-deficient culture solution inoculated with *Rhizobium* developed very poorly with many necrotic lower leaves. Blooming and formation of seeds took place relatively normally. Although the nodules were small they were able to supply their hosts with nitrogen.

The effect of boron on the growth and nitrogen fixation of pea plants was investigated. In agreement with the results of Brenchley and Thornton with broad beans it was found that no nodules developed on the roots of peas growing in nutrient solutions in the absence of boron. Due to this the plants died from nitrogen deficiency. Supplied with small amounts of boric acid normal nodules developed, which fixed nitrogen independently of the fact that pronounced symptoms of boron deficiency became apparent at an early date in the tops of these plants. To obtain normal plants considerably higher amounts of boric acid had to be applied.

Supplied with combined nitrogen the pea plants required somewhat higher amounts of boron than in the absence of this compound, probably due to a higher production of dry matter.

In an experiment with sand-peat mixtures pea plants supplied with potassium nitrate required considerably higher amounts of boron than plants supplied by the nodules. Presumably the more alkaline reaction of the peat in the former case had decreased the availability of the boron.

In experiments with natural soils a response of the pea plants to boron was found in some cases. In contrast to the experiments with culture solutions no symptoms of B-deficiency were seen in the tops of these plants. The fixation of nitrogen was decreased, however, and due to this the plants ripened at an earlier date and gave lower yields.

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